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Managing Editor

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Article No. ent.27301

Synthesis of adult accessory gland secretory proteins corresponds with the gene dose: a study with the hybrids of *Drosophila nasuta* subgroup

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ABSTRACT: The expression levels of different autosomal and X-linked accessory gland secretory protein fractions were examined in the hybrids of *D. n. nasuta, D. n. albomicans, D. n. kepulauana, D. s. albostrigata* and *D. s. neonasuta* in comparison with the parent species. The analysis showed that the autosomal fractions express half the parental quantity while the X-linked fractions reach parental levels.

KEYWORDS: *Drosophila*, male accessory gland, X-chromosome, gene expression, *nasuta*

INTRODUCTION

There are two accessory glands in the adult male's reproductive system of *Drosophila*. They develop from a special set of cells in the genital imaginal disk, upon instruction by genes that determine the sexual phenotype of the animal (Wolfner, 1997). These accessory glands synthesize and secrete a complex mixture of proteins, carbohydrates, lipids and amino acids (Chen, 1984) that are tissue specific, age specific, sex specific (Chapman and Wolfner, 1988) and are concerned with reproduction (Chen, 1996). By way of densitometry, Stumm-Zollinger and Chen (1988) have studied the expression of *Acp* genes in terms of rate of synthesis and accumulation of species specific fractions in the interspecific hybrids of some species of *D. melanogaster* subgroup. They have demonstrated that the synthesis of species specific secretory protein fractions are half in their quantities as compared with those of the parents and attributed these differences to difference in gene dose. They however, could not analyze the expression of X-linked fractions since all the 36 *Acp* genes in *D.*

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melanogaster are localized in autosomes and none on sex chromosomes (Wolfner *et al.*, 1997).

In different species of *D. nasuta* subgroup that show different degrees of reproductive isolation (Wilson *et al.*, 1969), we have recently identified accessory gland protein fractions that show autosomal and X-linked pattern of inheritance (Ravi Ram and Ramesh, 1999, 2001). In the light of these findings, present investigation was carried out to determine the extent of synthesis of X-chromosomal and the autosomal protein fractions in different hybrids.

MATERIALS AND METHODS

Stocks of *D. nasuta nasuta* (Coorg, India; Stock No. 201.001), *D. n. albomicans* (Okinawa, Japan; Stock No. 202.002), *D. n. kepulauana* (Sarawak, Stock No. 203.001), *D. sulfurigaster albostrigata* (Cambodia; Stock No. 207.001) and *D. s. neonasuta* (Mysore, India; Stock No. 206.001) were obtained from Drosophila Stock Center, Department of Studies in Zoology, University of Mysore, Mysore, India. All these stocks were cultured and maintained at 22 ± 1 °C on standard wheat cream agar medium seeded with yeast. Synchronized eggs collected from these stocks by modified Delcour technique (Ramachandra and Ranganath, 1988) were allowed to develop under uniform conditions of temperature, space, food, moisture and larval density. Unmated males and virgin females were isolated from the above-mentioned cultures within 2–3 hr, of their eclosion from the pupal case and were transferred to separate vials (8 × 2.5 cms) containing fresh media. They were aged for 5 days before conducting reciprocal crosses between compatible members to get the F_1 generation. Unmated parental as well as F_1 males were isolated and the accessory gland secretory protein samples were prepared as described earlier (Ravi Ram and Ramesh, 1999). The protein quantities in these samples were estimated by way of micromethod (Neuhoff, 1985). We used 10 μg of protein from the samples thus prepared for electrophoretic separation on 13.4% SDS-polyacrylamide gel. After overnight fixation in prestaining solution, the gels were stained and destained for 3 hr, each in Coomassie Brilliant Blue R-250 and destaining solution (Ravi Ram and Ramesh, 1999). The quantities of the specific protein fractions on the gels were analyzed to obtain optical density (OD) values through volume analysis mode in Molecular Analyst software of the Gel documentation system (Gel Doc 1000, Bio-Rad, USA). Student *t*-test was applied to check the level of significance with respect to the differences in OD values of autosomal and X-linked fractions between parents and the hybrids.

RESULTS AND DISCUSSION

The absorbance curve of CBB R-250 as a function of the amount of protein is shown to be linear (Fishbein, 1972; Kahn and Rubin, 1975). The Gel Doc 1000 (Bio-Rad, USA) gel documentation system used in the present investigations for image analysis comprises light detectors that are capable of converting biological signals into digital data. With white light transilluminator, the intensity of various signals (i.e. bands or

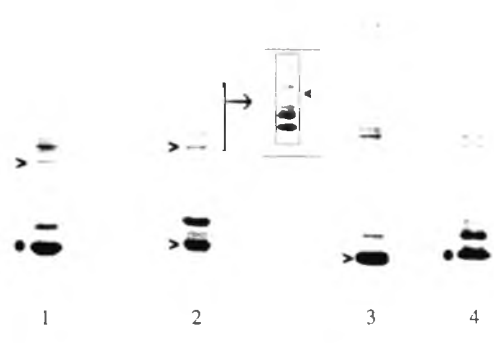

FIGURE 1. Showing X-linked and autosomal accessory gland secretory protein fractions in different members of *nasuta* subgroup. (1) *D. n. nasuta* (2) *D. n. albomicans* (3) *D. n. kepulauana* (4) *D. s. albostrigata* > X-linked fractions, • Autosomal fraction. Inset: silver stained pattern of *D. n. albomicans* showing 55 kD fraction.

spots on a gel) would be directly proportional to the intensity of gray levels displayed on the computer monitor, which could be measured in terms of OD. Quantitative estimation is done using volume analysis mode of Molecular Analyst software, which fully exploits the three-dimensional character of the digitized data. The value of OD/mm in terms of absolute quantitation would be equivalent to concentration.

Table 1 embodies the data on different fractions and their OD values in parents and hybrids. The difference in the OD values of 26 kD fraction of *D. n. nasuta* and 25 kD fraction of *D. s. albostrigata* between parent and hybrid was found to be significant; whereas the difference in the OD values of 55, 40 and 25 kD fractions of *D. n. albomicans*, 40 kD fraction of *D. n. nasuta* and 24 kD fraction of *D. n. kepulauana* was found to be non significant. Figure 1 shows different fractions that are under consideration in the present study.

SDS-PAGE pattern analyses of the male accessory gland secretions in different members of the *nasuta* subgroup, in the F_1, F_2 and backcross progenies of interspecific crosses have demonstrated that the 55, 40 and 25 kD protein fractions of *D. n. albomicans*, 40 kD fraction of *D. n. nasuta* and 24 kD fraction of *D. n. kepulauana* show X-linked pattern of inheritance; while the 26 kD fraction of *D. n. nasuta* and 25 kD fraction of *D. s. albostrigata* follow autosomal pattern of inheritance (Ravi Ram and Ramesh, 1999, 2001).

The males of *Drosophila* have two major sets of autosomes (homozygous), one X-chromosome and one Y-chromosome (hemizygous). The interspecific hybrid males would have one set of autosomes and the X-chromosome inherited from the female parent and the other set of autosomes and Y-chromosome inherited from the male parent. Hence, in the F_1 hybrids, the genes of one species are represented in only one dose. Stumm-Zollinger and Chen (1988) showed that the autosomal *Acp* genes coding for proteins specific for one or the other parent species are expressed normally in the hybrid cells with the rate of synthesis and accumulation being half when compared

TABLE 1. Different accessory gland secretory protein fractions and their OD values in parents and hybrids

Species/Subspecies	Fraction	Chromosomal linkage	OD values		Parentage	t a/b
			Parent (a)	Hybrid (b)		
D. n. nasuta	26 kD	Autosomal	20.01 ± 0.25	12.41 ± 0.06		24.08*
	40 kD	X-linked	3.20 ± 0.14	3.08 ± 0.12		0.486
D. n. albomicans	25 kD	X-linked	9.57 ± 0.11	9.19 ± 0.07	*D. n. nasuta* X *D. n. albomicans*	2.42
	55 kD	X-linked	5.56 ± 0.22	4.56 ± 0.22		2.13
	40 kD	X-linked	3.40 ± 0.10	3.33 ± 0.09		0.431
D. n. kepulauana	24 kD	X-linked	21.18 ± 0.23	20.17 ± 0.44	*D. n. nasuta* X *D. n. kepulauana*	1.84
D. s. albostrigata	25 kD	Autosomal	8.13 ± 0.19	4.75 ± 0.14	*D. s. albostrigata* X *D. s. neonasuta*	11.26*

Data are presented as mean ± SE; * $P < 0.001$; df = 4.

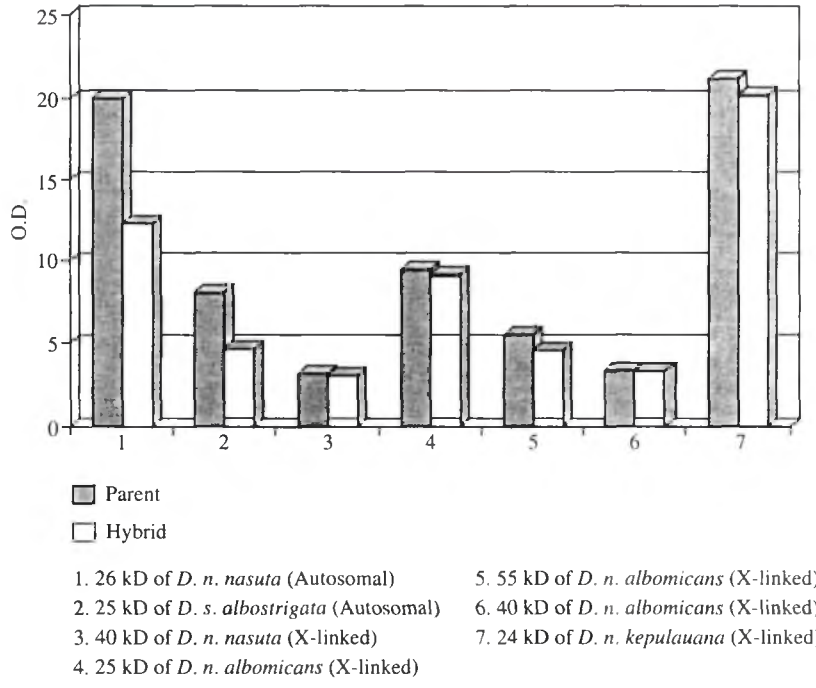

FIGURE 2. O. D. Value of X-linked and autosomal fractions in parents as well as in hybrids.

with the homozygous parent species, thus corresponding to the gene dose. In the present study, the OD values for 26 kD fraction in the hybrids of *D. n. nasuta* X *D. n. albomicans* and the OD values of 25 kD fraction in the hybrids of *D. s. albostrigata* X *D. s. neonasuta* are found to be nearly half when compared with that of their parents irrespective of the direction of the cross (Table 1; Fig. 2). As the genes coding for 26 kD fraction in *D. n. nasuta* and 25 kD in *D. s. albostrigata* are autosomal, in the parent they exist in double dose; while in the hybrid they are present in single dose and hence, the reduction in the OD values. However, the fractions that show X-linked pattern of inheritance *viz.*, 25, 55 and 40 kD fractions in *D. n. albomicans*, 40 kD fraction in *D. n. nasuta* and 24 kD fraction in *D. n. kepulauana* have almost similar OD values in the parents and in the F_1 (Table 1; Fig. 2) since there will not be any difference in the gene dose as males of both the parent species and the hybrids have only one X-chromosome. Therefore, in the hybrids, the *Acp* genes that are autosomal synthesize only half the quantity as compared with that of the parent and the X-chromosomal genes show full expression by synthesizing proteins to reach the parental levels. As a further step, we have analyzed one autosomal fraction (26 kD) and an X-linked fraction (25 kD) from day 1 to day 7 to determine the pattern of their synthesis in the F_1 males of *D. n. nasuta* X *D. n. albomicans* when their gene dose is same. Through such an analysis we have found that the hybrids, the synthetic pattern

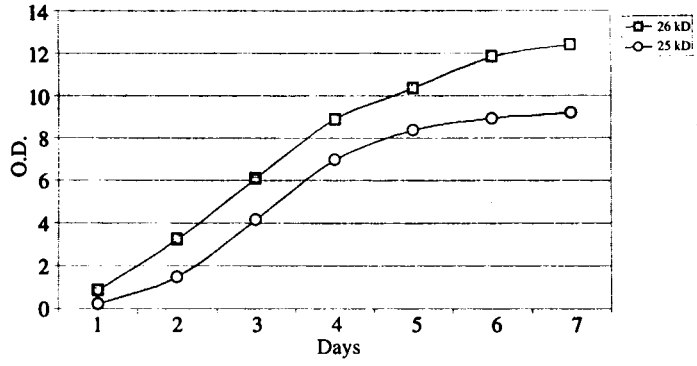

FIGURE 3. Synthetic pattern of an autosomal and a X-linked fraction during aging of the hybrid male.

of autosomal and X-linked fractions is very much similar (Fig. 3). This suggests that their expression is regulated in a similar manner. Hence, the differences/similarities in the quantities observed are because of differences/similarities in gene dose. Thus, the present study while supporting the findings of Stumm-Zollinger and Chen (1988) with regard to the autosomal gene expression has also provided an insight in to the nature of expression of X-chromosomal *Acp* gene(s).

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Biology and nutritional efficiency of flea beetle, *Altica himensis* Shukla (Coleoptera: Chrysomelidae) and its probable role as a biological control agent of weed in Kumaon Himalayas

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ABSTRACT: Life history studies on flea beetles, collected from Oak mixed forest of Kumaon Himalayas for two years, 1994 and 1995, are reported. Both, adult and larvae attacked herbs belonging to *Impatiens*, *Rumex* and *Oenothera*, growing luxuriantly in the forest particularly near the man made clearings. There are three larval instars. Duration, biomass and body measurements of juvenile stages and adults are reported. Total larval and pupal duration recorded 22.2 and 9.4 days respectively with a prepupal duration for 7.6 days. Longevity of adults is 46.8 and 44.6 days for female and male respectively. One generation is completed in 84 to 90 days. Larvae and adults attacked the tender leaves, petioles, stem and young inflorescence, thus causing considerable damage. None of the useful plants are found to be attacked. Nutritional efficiency of the beetle was also studied. Approximate digestibility (AD), tissue growth (ECD) and ecological growth efficiencies (ECI) recorded are c.a. 59.7, 15.9 and 9.4% respectively. To evaluate the potentialities of *Altica himensis* as a biological control agent of weed, 'Revised Harris-Scoring-System' was applied. A total of 32 points were scored by the beetle, declaring it as a partial biological control agent.

KEYWORDS: Biology, Nutritional-efficiency, Harris scoring system, weed, flea beetle, biological control

INTRODUCTION

Altica species are distributed throughout the world. These are phytophagous, attacking crops, vegetables, fruits, ornamentals and forest trees. Many of the species are reported to control the weed plants. Shah and Jyala (1998) reported *Altica himensis* from weed plants in the forests. Notable contributions on the life history of flea beetles were made by Gibson (1913); Zwolfer (1965); Mineo and Iannazzo (1986); Nayek and Banerjee (1987). Kannan and Anand (1992) described the genitatic structure in *Altica* Muller. It is the first record on biology, nutritional efficiency etc. of *A. himensis*.

TABLE 1. Duration, biomass and measurements of various instars (mean ± SE) of *A. himensis*

Stage	Duration (days)	Biomass (mg)	Head width (mm)	Body length (mm)
1st Instar	4.6 ± 0.24	0.35 ± 0.13	0.31 ± 0.01	2.82 ± 0.11
2nd Instar	5.4 ± 0.25	1.02 ± 0.07	0.45 ± 0.01	4.06 ± 0.06
3rd Instar	12.2 ± 0.37	3.44 ± 0.067	0.72 ± 0.02	8.70 ± 1.12
Pupa	9.4 ± 0.25	5.00 ± 0.95	—	3.20 ± 0.06
Male	44.6 ± 1.02	3.68 ± 0.058	1.00 ± 0.02	4.59 ± 0.55
Female	46.8 ± 1.15	4.64 ± 0.14	1.20 ± 0.01	5.24 ± 0.112

Most of the current studies on *Altica* species revolve round its exploitation as a biological control agent of weeds in rice fields (Nayek and Banerjee, 1987; Ooi, 1987; Shui, 1990). Slansky and Scriber (1982); Kaushal and Kalia (1991) have studied the nutritional ecology of beetles.

Harris (1973) proposed a scoring system (Table 2) to serve as a simple method for recognizing 'effective' biological control agents, or at least for eliminating unpromising candidates. Goeden (1983) revised this system.

TABLE 2. A proposed revision of the scoring system of Harris (1973) for determining the potential effectiveness of a species of phytophagous insect as a candidate for the biological control of weeds (candidates to receive one score, the most applicable, under each criterion)

I.	Initial Assessment of Destructiveness in Native Range		
	(1)	Direct damage inflicted under field conditions	
		(A) Leaf mining	[1]
		(B) Sucking damage or gall-formation	2
		(C) Defoilation or phytotoxin-enhanced sucking damage	4
		(D) Destruction of vascular or mechanical support tissues as an endophage	6
		(E) Substantial seed destruction	
	-	Long-lived perennials	1
	-	Short-lived plants not dependent on reproduction by seeds	2
	-	Annuals or biennials only reproducing by seeds	6
	(2)	Indirect damage inflicted	
		(A) None	0
		(B) Limited	[2]
		(C) Host plant consistently rendered susceptible to attack by secondary insects of phytopathogens	4
		(D) Vector or virulent phytopathogen	6
	(3)	Phenology of attack	

TABLE 2 continued

	(A)	Limited period of attack not increasing plant susceptibility to drought, fros, or competition from other plants	1
	(B)	Limited period of attack by seed-damaging insects not covering entire reproductive period of the weed	2
	(C)	Limited period of attack, but increasing host-plant susceptibility to frost, drought, or competition from other plants	4
	(D)	Prolonged attack covering the entire growing season or reproductive period of annual weed by seed-damaging insect	[6]
(4)	Number of generations		
	(A)	Obligate univoltine species	0
	(B)	Two or three generations a year, climate permitting	[2]
	(C)	Four or more generations a year, climate permitting	3
(5)	Number of progeny per female per generation		
	(A)	<500	[0]
	(B)	500–1000	2
	(C)	>1000	3
(6)	Extrinsic mortality factors		
	(A)	Natural control largely effected by non-specific enemies or abiotic ecological factors	[0]
	(B)	Subject to extensive mortality from competitors for the host plant, combined with a common occurrence	3
	(C)	Subject to extensive mortality from specialised enemies including diseases, and relatively immune to not-specific enemies	6
(7)	Feeding behaviour		
	(A)	Solitary feeders (cannibalism, avoidance or other traits precluding immatures or adults from feeding in close proximity)	0
	(B)	Gregarious or colonial feeders (intrinsic behaviour not precluding immatures or adults from feeding in close proximity)	[3]
(8)	Distribution		
	(A)	Local only	0
	(B)	Covers about half of the range of the target weed	2
	(C)	Covers about three-quarters of the range of the target weed	4
	(D)	Covers the full range of the target weed	[6]
II.	Suitability as a Biological Control Agent		
(1)	Host-plant source of insect		

TABLE 2 continued

	(A)	Obtained from different host-plant genus than the target weed (oligophagous)	2
	(B)	Obtained from a host plant belonging to the same genus but not the same species as the target weed	4
	(C)	Obtained from the target weed species as a host plant	6
(2)	Ease of culture		
	(A)	Culture impossible; work must proceed with field-collected specimens	0
	(B)	Culture difficult on target weed or possible only on restricted developmental stage of living host plants	2
	(C)	Culture easy on artificial diet or readily propagated host plants, including target weed	4
(3)	Potential safety		
	(A)	Reported infesting a useful plant	**0**
	(B)	Not known as a plant pest, but cogeneric with pest species 2	2
	(C)	Unreported as a plant pest; no plant pest in same genus or higher taxon	6
(4)	Host-plant specificity		
	(A)	Readily feeding on critical test plant under confined laboratory conditions	6
	(B)	No feeding on critical test plant in laboratory	**6**
		Total 32 points	

MATERIALS AND METHODS

The study-site, a mixed oak forest was selected near D.S.B. Campus, Nainital (29°23′N and 79°28′E, altitude). Weather data was obtained from the nearest meteorological station, state Observatory, Nainital. Seasonal variation in temperature, humidity and annual rainfall was recorded four two years i.e. 1994 and 1995.

The life-history of *Altica himensis* was studied in the laboratory by rearing larvae (n = 20–30/jar) on fresh leaves of *Impatiens amphorata* in glass jars (7 × 18 cm). The jars were sterilised and rinsed in 95% ethanol and were lined with several layers of moist blotting paper in the bottom to supplement the moisture needed for insect development. To preserve the fresh state of leaves, a wet cotton swab was wrapped around excised branches. Uneaten leaves, faeces, and dead larvae were removed daily from the jars. The jars were regularly checked for exuviae and head capsules to determine the number and duration of larval instars. Larval growth was followed and recorded by measuring the head width any body length of larvae (n = 30) that were in-between moults with the help of a microscope with micrometer. The weight gain was measured by a single pan-electronic balance with 0.1 mg accuracy. Larvae were allowed to feed

on host leaves until pupation. The fully fed larvae were transferred to similar container, furnished with 1/2″ layer of loose moist soil into which pupation took place. Pupae were kept in separate empty jars for recording adult emergence and sex ratio. These adults were maintained for mating, oviposition and longevity studies in 7 × 8 cm jars.

For the quantitative estimation of food consumption, the leaves from experimental plants were cut into 1 square inch sections, weighed and fed to 100 newly hatched first instar larvae held in groups of 10 and 20, second and third instar larvae were held individually. Same amount of leaf was oven dried at 80 °C to measure the initial weight of food. The live weight of each larva or group of larvae in each instar was determined before feeding (initial weight). After 24 hours of feeding the larvae were reweighed (final weight). The uneaten food and egesta (frass) were collected and oven dried at 80 °C to a constant weight. Dry weight, equivalents of adult and larvae were also calculated by oven drying 50 adults and 100 larvae of same age group. Ecological efficiencies were calculated using Waldbauer's (1968) expression:

(i) Approximate digestibility (AD) or Assimilation efficiency $= \frac{\text{Assimilation}}{\text{Consumption}} \times 100$

(ii) Tissue growth efficiency (ECD) $= \frac{\text{Tissue growth}}{\text{Assimilation}} \times 100$

(iii) Ecological growth efficiency (ECI) $= \frac{\text{Tissue growth}}{\text{Consumption}} \times 100$

Assimilation = Weight of food ingested-weight of faeces

Tissue growth (weight gained) = Initial weight – Final weight.

To assess the damage caused to the host plants, 'feeding-trials' were conducted in the laboratory on leaves of different age categories *viz.*, young, intermediate and mature during the 'Choice test' (Shah and Jyala, 1998). Investigations were made at twenty four hour interval to look for the recent feeding marks. An older feeding marks can be distinguished by brown-rim round the mark. To assess the effectiveness of *Altica* as a potential biocontrol agent of weeds, a careful rating of all biological characteristics was done by following 'Revised Harris Scoring systems' (Table 2). The points scored by the beetle, added together to declare it non effective/partially effective/fully effective biocontrol agent.

RESULTS

Seasonal variations in temperature and humidity were significant. The minimum temperature and humidity varied 2.8°C–21.2°C and 20.9–78.3% respectively. The maximum temperature and humidity ranged 12.6°–29.4° and 43.5–93.7%. The total annual rainfall being 2233 mm during the study periods. The life history studies have shown a complete synchronization of beetle life cycle with phenology of host plants.

The diapausing beetles become active from the first week of March and colonised on *Rumex hastatus* weed (Shah and Jyala, 1998). Soon copulation started. Its a long

and complex process lasted for 3–10 hr. After a preoviposition period of 7 ± 1.2 days eggs were deposited on ventral surface of leaf at intervals in installments. A mean of 7.6 eggs/mass (range 8–10, $n = 50$) and 6–18 masses/female were deposited. Thus 60–100 eggs (mean 75.6 ± 0.25)/female were laid. The egg is small oval and creamy white with slight longitudinal depression, in the middle having a black thread like mass by which one egg remained attached with the other and measured 0.68 ± 0.01 × 0.40 ± 0.04 mm. Incubation period recorded 5.2 ± 0.37 days (5–6 days).

Early larvae were gregarious and commenced feeding on leaves soon after hatching. Larval-cannibalism at earlier stage was common among siblings. The larvae passed through three instars. Duration of larval instars, biomass and body measurements are given in Table 1. The 1st and the 3rd instar had the shortest (4.6 ± 0.24 days) and longest (12.2 ± 0.37 days) duration, respectively. Maximum biomass (3.44 ± 0.06 mg) recorded at 3rd instar. These was a regular procession (1 : 1.4) seen in the increase of the head width throughout the larval development but it remained constant for a particular stage of development (Subha Rao and Abu Bucker, 1975).

Mature 3rd instar larvae left the host plant and moved into loose moist soil (1–2 cm deep) where pupation took place over a 7.8 ± 0.3 days. Dehiscence took place along the mid-dorsal thorax, and exuviae gradually slipped behind, liberating the pupa. Pupae are yellowish but darkened within 2–3 days. The adults emerged between 9–12 days.

Female is bigger (5.24 ± 0.1 mm) and male being smaller in size (4.5 ± 0.5 mm). Aedeagus measured 1.16 × 0.4 mm and sex-ratio was 1 : 1.55 i.e. females outnumbered the males. Longevity of females was also greater (46.8 ± 1.15 days) than males (44.6 ± 1.02 days).

The total duration from egg to adult took 43–49 days. One generation is completed in 84–90 days and three complete overlapping generations were recorded in one year. Graphic representation of life cycle is given in Fig. 1.

Results of the 'feeding trial' on *Altica himensis* are shown in Fig. 2. *Impatiens amphorata* suffered the most, 41.2 ± 2.1% young leaves, 35.0 ± 1.95% intermediate leaves and 9.75 ± 2.3% mature leaves were damaged. Young leaves of *Impatiens scabrida* also suffered a considerable damage (31.5 ± 1.0%), whereas mature leaves of most of the host plants were not selected by the beetle population. *Rumex nepalensis* suffered the negligible damage. About 5% young inflorescence in *Impatiens* species suffered the damage in absence of preferred foliage.

Results of nutritional efficiency have shown that initial biomass increased from 0.35 ± 0.13 mg (Ist instar) to 3.44 ± 0.06 mg in final instar followed by a sudden rise in pupal stage (5.0 ± 0.95 mg) indicating a build up of energy reserves for this non-feeding stage. There is a marked decline in biomass (3.68 mg) of adult due to utilisation of energy reserves of pupae for various developmental processes. Similarly the consumption increased from 0.90 to 3.02 mg/insect/day. A positive significant correlation was obtained between consumption and biomass ($r^2 = 0.811$); between consumption and egestion ($r^2 = 0.827$) and between consumption and tissue growth ($r^2 = 0.906$). A total of 8.50–10.65 mg dry weight of leaf was consumed per larva upto pupation. Efficiencies of food utilization by *Altica himensis* are shown in Table 3.

FIGURE 1. Graphic representation of life-history of *A. himensis.*

TABLE 3. Efficiencies of food utilisation in *A. himensis*

Stage	Approximate digestibility (AD)%	Tissue Growth (ECD)%	Ecologial Growth (ECI)%
1st Instar	62.20	14.85	9.22
2nd Instar	60.00	17.07	10.24
3rd Instar	57.62	16.50	9.52
Adult	56.84	15.24	8.66
Mean	59.17	15.91	9.41

Highest approximate digestibility (AD) was obtained in Ist instar which decreased in later stages of development; but efficiency to utilise the digested food/ingested food for tissue growth (ECD/ECI) increased from Ist instar to final instar.

Results of 'Revised Harris Scoring-System' are presented in Table 2. A sum of 32 points scored by *A. himensis* declares it as a partially effective biocontrol agent.

DISCUSSION

Results of the life-history of *Altica himensis* were compared with the findings in *Altica chalybea* (Gibson, 1913); *A. carduorum* (Zwolfer, 1965); *A. ampelophaga* (Mineo and Iannazzo, 1986) and *A. cyanea* (Nayek and Banerjee, 1987). However these values

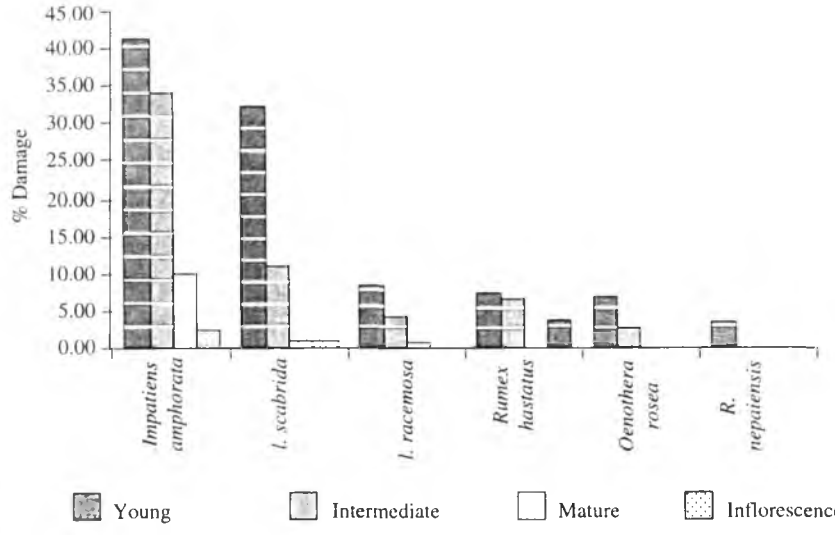

FIGURE 2. Percent damage caused to leaves of different ages by *A. himensis*.

were found close to *A. cyanea*, only with body-measurements and other observed, values being lower and total juvenile duration being longer in *A. himensis* (c.f. *A. cyanea*).

Most of the studies on *Altica* species revolve round its exploitation as a biological control agent of weeds. In rice growing countries *Altica cyanea* is found to control a number of weeds in rice-fields (Dubey, 1981; Nayek and Banerjee, 1987; Shui, 1990). However literature on the quantitative estimation of weed control is inadequate. Ooi (1987) calculated the percent damage caused to a weed *Melastoma malabathricum*. He observed that 12.1% plants were severely damaged, 48.3% slightly, 22.4% moderately damaged and 17.3% were without visible damage.

Thomas (1987) also observed that leaf age is more important than plant species in host plant selection and assessed the percent damage as 42.5, 31.1 and 4.1% caused to young, intermediate and mature leaves.

Latheef and Harcourt (1972); Slansky and Scriber (1982); Kaushal and Kalia (1991) have compiled the literature on quantitative measurements of food consumption and utilization for growth by insects. Range of ECD in coleopterans is reported to be 2 to 59% where as AD values ranged from 58 to 95%. ECI values increased from 5.6% (Ist instar) to 16.18% in IIIrd instar in oak weevil. The ECI and ECD bear inverse relationship to AD from Ist instar to adult (Kaushal and Kalia, 1991). Efficiencies of food utilisation in *A. himensis* are with in the reported range.

For screening the efficiencies of an insect as a biocontrol agent, a simple and remarkable 'scoring-Table' has been proposed by Harris (1973), revised by Goeden (1983). This table evaluates fifteen biological characteristics of biocontrol agents and assigns -12 to $+12$ scores to them. By adding these scores, the efficacy of a biocontrol

agent can be predicted, shortly before, during or after its initial release into the area of introduction. Goeden (1983) proposed to assess the effectiveness of biocontrol agents into three phases. Phase Ist and IIIrd are applied in field before exhaustive host specificity testing and detailed life history studies of selected insect species begin. The IIIrd phase of scheme is applied just before, during or shortly after the agent is released in some new area of same ecoclimatic conditions. According to Goeden (1983) a prospect scoring 20 points is considered as an ineffective agent, between 20 and 50 points is a partially effective and a score above 50 points is an effective biocontrol agent. A sum of 32 points scored by *A. himensis* declares it as a partially effective biocontrol agent.

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Determination of LD_{50} and LT_{50} of *Paecilomyces farinosus* (Holmskiold) Brown and Smith on larval instars of *Plutella xylostella* L.

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ABSTRACT: The lethal dose (LD_{50}) and lethal time (LT_{50}) of the entomopathogenic fungus, *Paecilomyces farinosus* against larval instars of *Plutella xylostella* was determined in the laboratory. The LT_{50} of the disease when infected with a dosage of 1.7×10^8 spores/ml concentration against larval instars, ranged from 2.12 days for I instar to 3.31 days for IV instar larva. The LT_{50} increased as the instar of the larva advanced. When different dosages (1.7×10^2 to 1.7×10^{10} spores/ml) were tested against II instar larva, the LT_{50} decreased as the dosage was increased. Thus, LT_{50} was 5.18 days with the lowest dosage and 2.31 days for the highest dosage tested. However, the median lethal dose of the fungus (LD_{50}) against II instar larva of *P. xylostella* was 1.114×10^5 spores/ml.

KEYWORDS: *Paecilomyces farinosus*, *Plutella xylostella*, LD_{50}, LT_{50}

INTRODUCTION

Paecilomyces farinosus, an entomopathogenic fungus is considered to be a potential biological control agent of many insect pests (Prenerova, 1994) and it was recently isolated from the field population of *Plutella xylostella* on cabbage (Gopalakrishnan, 1999). A laboratory experiment was carried out in order to determine the lethal dose (LD_{50}) and lethal time (LT_{50}) of the fungus against the larval instars of *P. xylostella*. The results of the experiments are reported in this paper.

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MATERIALS AND METHODS

Effect of dose on LT_{50} of *P. farinosus* on different instars

A test was conducted to determine LT_{50} of fungus in all the four larval instars of *P. xylostella*. The larval instars used were from the insect culture reared on cabbage leaves in the laboratory. The fungus, *P. farinosus* initially isolated from *P. xylostella* and grown on Sabouraud maltose agar + yeast (SMAY) slants was utilised for inoculation of larvae. The fungal spore suspension was obtained form the culture maintained on SMAY slants. The spores were washed with sterile distilled water + Triton *x*-100 (0.01%) with a sterile glass rod and the suspension filtered through double layered muslin cloth. The stock suspension of 1.7×10^{10} spores/ml was diluted to 1.7×10^8 spores/ml for inoculating I–IV larval instars of *P. xylostella*. Counting of the spores was made using double ruled improved Neobauer haemocytometer under phase-contrast microscope.

Twenty larvae in each instar were treated with a dose of 1.7×10^8 spores/ml and each treatment repeated thrice. The larvae were dipped in the suspension with the help of forceps and camelin hair brush. Excess suspension drained off by mearly touching the larvae on the Whatman filter paper. The inoculated larvae were placed on tender cabbage leaf with petiole wrapped in cotton swab, in a plastic container of size 13.5 × 11.0 cm. The container was provided with moist cotton layer covered with tissue paper at the bottom of the container to provide humidity. The container was covered with meshed lid to provide aeration to the larvae. Another set of twenty larvae of each instar, treated similarly with out the spores served as control.

The experiments were conducted at room temperature (27±2 °C). Daily observation on larval mortality was recorded. The dead larvae which showed mycelial growth was considered as death due to fungal infection.

The LT_{50} of the dose of fungal spores to kill the different larval instars was assessed in hours following Biever and Hostetter (1971).

$$LT_{50} = a + e\frac{(c - b)}{D}$$

where, a = the number of hours from the initiation of the test until the reading made just before the 50% value was recorded, b = the total number of larvae dead at the reading just before 50% value was reached, c = 50% of the total number tested, d = the number of larvae dying in 24 hr period during which the 50% mortality was reached, and e = the number of hours between mortality counts.

Determination of LD_{50} and LT_{50} of the fungus in II instar larvae of *P. xylostella*

To determine LD_{50} and LT_{50} of *P. farinosus* in II instar larvae of *P. xylostella*, 10 different spore concentrations were used *viz.*, 1.7×10^{10}, 1.7×10^9, 1.7×10^8, 1.7×10^7, 1.7×10^6, 1.7×10^5, 1.7×10^4, 1.7×10^3, 1.7×10^2 and 1.7×10^1 spores/ml. The preparation of spore suspension, fixing of dose and inoculation of larvae were as described earlier. Sixty II instar larvae were inoculated for each spore concentration with three replicates, each replication having twenty larvae. Similar number of larvae

TABLE 1. Effect of the fungus at 1.7×10^8 spores/ml on the LT_{50} against larval instars of *P. xylostella*

Instar	LT_{50} (days)	% Mortality
I	2.12	100.0
II	3.12	90.0
III	3.25	90.0
IV	3.31	80.0

with similar replication treated with 0.01% Triton x-100 without the spores served as control. Larvae after treatment kept on cabbage leaves in plastic containers as described earlier.

The experiment was conducted at room temperature at 27±2°C. Observations taken for mortality of larvae at every 6 hr intervals till 108 hr after inoculation. The dead cadavers were kept on moist filter paper in Petri dishes to assess the fungal mortality. The dose-mortality data were subjected to probit analysis (Finney, 1962) for LD_{50} and to Biever and Hostetter (1971) method for LT_{50}. The data on percentage larval mortality (II instar) for different doses was transferred to arc sine values and analysed statistically.

RESULTS AND DISCUSSIONS

Effect of dose on the LT_{50} of fungus in different instars

The median lethal time (LT_{50}) and percent mortality of the different instars of the larvae are presented in Table 1.

It is evident from the Table 1 that the LT_{50} increased as the instar of the larva advanced. Thus, from 2.12 days for the first instar larva, the LT_{50} upto 3.31 days for the fourth instar larva. As the instars advanced, a decrease in mortality and an increase in time for initial mortality were recorded. Further, the first instar larvae were highly susceptible to the fungus recording total mortality, followed by second and third instar larvae by recording 90 per cent mortality. The fourth instar larvae were comparatively less susceptible recording 80 per cent mortality.

Effect of different doses of fungus on the LT_{50} of second instar larva

To determine the effect of dosage of the fungus on the LT_{50}, ten different concentrations ranging from 1.7×10^1 to 1.7×10^{10} spores/ml were inoculated to second instar larvae.

The per cent mortality and LT_{50} of the larvae when inoculated with different dosages are presented in Table 2. The mortality rate increased with the dosage recording a minimum of 5.0 per cent at a dose of 1.7×10^2 and a maximum of 100 per cent at the highest and next highest dosages (1.7×10^{10}, 1.7×10^9), though LT_{50} differs for

TABLE 2. Effect of different concentrations of fungal spores on LT_{50} against second instar larvae of *P. xylostella*

Dosage (spores/ml)	LT_{50} (days)	% Mortality
1.7×10^{10}	2.31	100.0 (90.0)
1.7×10^{9}	2.68	100.0 (90.0)
1.7×10^{8}	3.12	90.0 (71.9)
1.7×10^{7}	3.37	55.0 (47.9)
1.7×10^{6}	3.75	40.0 (39.2)
1.7×10^{5}	4.29	30.0 (33.1)
1.7×10^{4}	4.68	25.0 (29.9)
1.7×10^{3}	4.93	15.0 (22.8)
1.7×10^{2}	5.18	5.0 (12.9)
1.7×10^{1}	—	00.0 (00.0)
Control	—	00.0 (00.0)
C.D. ($P = 0.05$)	—	03.8

(Figures in parenthesis are arc sine transformed values.)

these two dosages. The LT_{50} decreased as the dosage was increased. Thus, the LT_{50} was 5.18 days with the lowest dosage and 2.31 days for the highest dosage applied.

It was further noted in the present studies that lower concentration of fungus inoculum resulted in a longer LT_{50}. Devaprasad *et al.* (1990) reported that LT_{50} depended upon the size of inoculum and the lower the spore concentration the longer was the LT_{50}. Thus the LT_{50} ranged from 2.12 to 3.31 days for first to fourth instars (Table 1). The LT_{50} of *P. farinosus* differs from *P. xylostella* to any other insects. According to Litchfield (1969) the median lethal time (LT_{50}) of hyphal bodies of *P. farinosus* in *S. exigua* for external infection was 4.1 days and that of microfed hyphal bodies it was 6.3 days as cited by Agudelo and Falcon (1983). But the LT_{50} at various concentrations tested against II instar larvae of *P. xylostella* ranged from 2.31–5.18 days from the highest concentration of 1.7×10^{10} spores/ml to the lowest concentration of 1.7×10^{2} spores/ml (Table 2). As the concentration increased the rate of LT_{50} was not only lowest but the percentage mortality was also higher.

Probit analysis of dosage-morality response of second instar larvae of *P. xylostella* to *P. farinosus*

The results of the LD_{50} value determined through probit analysis are presented in Table 3 and illustrated in Fig. 1. Among the various estimate of the regression based probit analysis, the chi-square test of the bioassay showed homogeneity of the test population which is a reflection of a good fit of the observed and expected responses. The median lethal dose of the fungus was 1.114×10^{5} spores/ml for the second instar larva of *P. xylostella*.

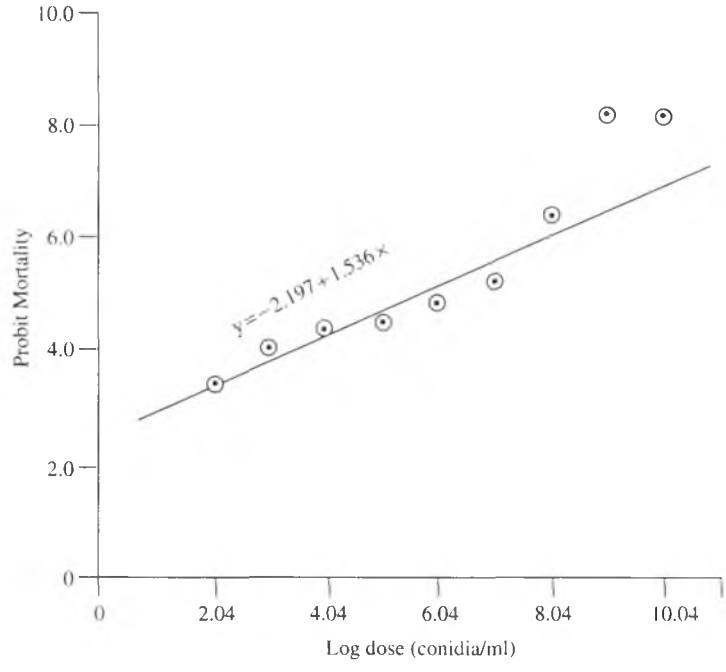

FIGURE 1. LD_{50} value for second instar larvae of *Plutella xylostella*.

TABLE 3. Effect of different concentrations of fungal spores on LD_{50} against second instar larvae of *P. xylostella*

Homogeneity	Regression equation	LD_{50} spores/ml	Fiducial limit (95% confidence)		Standard error
			Lower limit	Upper limit	
$X^2_{(8)} = 15.507$	$Y = 2.19690 + 1.53687X$	1.114×10^5	3.61×10^5	3.44×10^4	0.9552

The LD_{50} of the fungus against II instar larvae of *P. xylostella* was 1.114×10^5 spores/ml. The fiducial limit at 95% confidence was 3.61×10^5 and 3.44×10^4 spores/ml for lower and upper limit, respectively (Table 3). The LD_{50} of *P. farinosus* spores for second instar larvae of *H. armigera* was 5.2×10^9 spores/ml (Devaprasad *et al.*, 1990). The difference in LD_{50} for *H. armigera* and *P. xylostella* may be due to the size and difference in nutritional content of the insect.

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Blood meal induced ovarian proteins during gonotrophic cycles in malaria vector *Anopheles stephensi* (Diptera: Insecta)

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ABSTRACT: The qualitative and quantitative analysis of soluble proteins in ovaries of malaria vector, *Anopheles stephensi* in relation to three gonotrophic cycles during haematophagy and sugar feeding have been carried out by SDS-PAGE. In total 51 polypeptides of molecular weight ranging from 10.5 to 185 KDa were revealed in ovaries. Six polypeptides were synthesized in the ovaries immediately after blood feeding. The first and second gonotrophic cycles coincided with the synthesis of 4 and 2 additional polypeptides respectively. The decline in protein content (35%) after third gonotrophic cycle could be attributed to the degradative processes associated with senescence. In total, 21 polypeptides disappeared from the ovaries of sugar fed females during various stages as compared to blood fed. The comparative analysis of polypeptides pattern of various tissues during three gonotrophic cycles have indicated that these proteins seems to be involved in ovarian maturation.

KEYWORDS: Ovaries, protein, gonotrophic cycles, blood

INTRODUCTION

Oogenesis is one of the most interesting biological process in terms of development and physiology. An anautogenous mosquito requires one or more blood meals to produce a batch of eggs, the haemoglobin content of the blood meal providing the major protein source for the egg development (Briegel, 1985). Mosquitoes are known to adjust their egg-clutch size according to quality and quantity of the blood meal, small blood meals resulting in the resorption of some developing follicles (Clements and Boocock, 1984). Mosquito vitellogenesis has been studied comprehensively only in *Ae. aegypti* (reviewed by Raikhel, 1992). In parallel, digestion of the blood in the midgut activates the production of large amount of vitellogenins in the fat body, which evantually triggers the vitellogenic growth of the oocyte (Borovsky *et al.*, 1985).

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These physiological processes or characterization of ovarian proteins may present opportunities for novel methods of vector control.

No information seems to be available regarding the developmental pattern of ovarian polypeptides in mosquitoes in relation to gonotrophic cycles and different feeding habits. Therefore, the present study has been carried out with a view to examine the constitutive and blood meal induced polypeptides pattern during three gonotrophic cycles in ovaries of ageing mosquitoes and compared with other tissues like haemolymph (Gakhar and Shandilya, 2000) as well as in freshly laid eggs (Gakhar *et al.*, 1997). In addition, the changes in these polypeptides have also been examined in relation to sugar feeding. These proteins are the candidates to understand the final regulation of vitellogenins. The present investigations also complements the detailed temporal and spatial analysis of polypeptides in various tissues of *Anopheles stephensi*, a vector of malarial parasite on the Indian subcontinent where its control has been hampered by insecticidal resistance.

MATERIALS AND METHODS

Mosquito rearing

The Delhi strain of *A. stephensi* mosquitoes was maintained under standardized conditions at 28 ± 2 °C and relative humidity of 70–80% as described earlier (Gakhar *et al.*, 1997).

Sugar feeding/haematophagy

The mosquitoes exclusively maintained on sugar meal provided with 4% glucose solution and water soaked raisins were not given any blood meal. However, controlled mosquitoes were allowed to take normal blood meal.

Different developmental stages of known chronological age were taken after each gonotrophic cycle. The mosquitoes of same age group were divided into three (triplicate) groups and ovaries were collected from each set as described below.

Dissections for ovaries

The mosquitoes were knocked down before dissection. In a successful dissection, the ovaries along with the midgut were pulled out into a droplet of normal saline solution.

Analysis of soluble proteins

The soluble proteins were quantified by the method of Lowry *et al.* (1951). For qualitative analysis, 60 μg of protein, as extracted by the method already described (Gakhar *et al.*, 1997) was loaded in each well after mixing with equal volume of 2X gel loading buffer. The mixture was heated at 100 °C for 3–5 minutes before loading. The protein samples were stored at −20 °C until use for SDS-PAGE (Laemmli, 1970). The gels were silver stained as described by Morrissey (1981). The molecular weight of proteins was determined using markers (SIGMA).

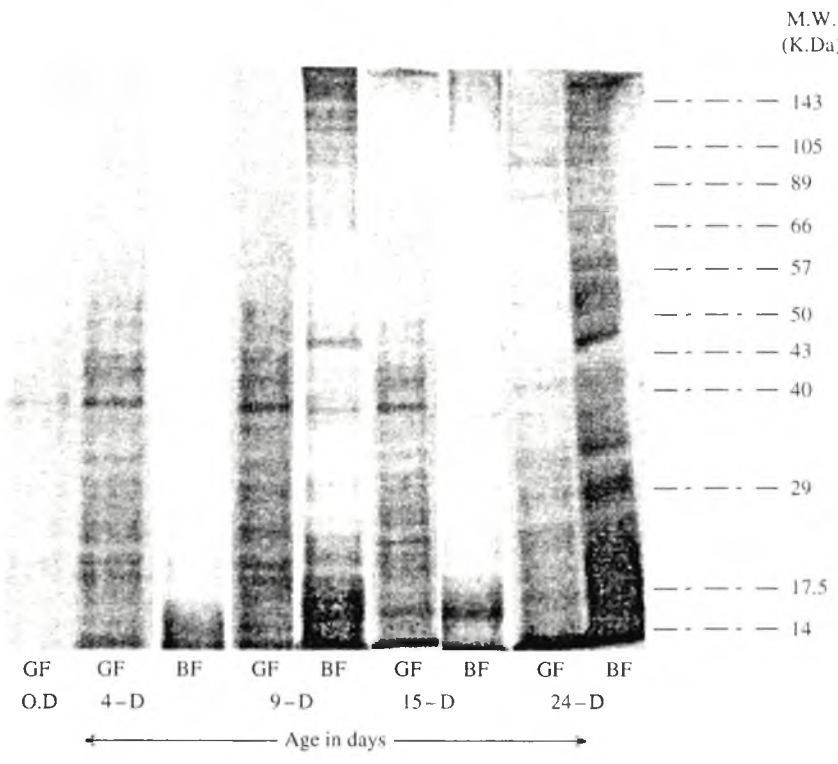

FIGURE 1. The pattern of polypeptides in the ovaries during development and ageing of *A. stephensi*. GF - Glucose feeding, BF - Blood feeding, D - Days after emergence, MW - Molecular weight.

RESULTS

The pattern of polypeptides in relation to three gonotrophic cycles in ageing mosquito, *A. stephensi* is presented in Fig. 1.

Ovaries of *A. stephensi* revealed 51 polypeptides in total of molecular weight ranging from 10.5 to 185 kDa in ageing mosquitoes when fed on blood. The first gonotrophic cycle coincided with the synthesis of 6 new polypeptides (58, 73, 91, 95, 109 and 116 kDa) on day-4 and 4 polypeptides (37, 100, 140 and 143 kDa) on day-9. However, 4 polypeptide (38.5, 44.5, 66 and 173 kDa) disappeared during first gonotrophic cycle. Similarly, 2 polypeptides (69 and 95 kDa) also disappeared after 2nd gonotrophic cycle in 15 days old females (Fig. 1). During third gonotrophic cycle, only 2 high molecular weight polypeptides (122 and 143 kDa) were synthesized on day-24 and 6 polypeptides (11.5, 18, 29, 30, 137 and 173 kDa) disappeared.

The quantity of soluble proteins in the ovaries was about $1.50 \pm 0.30\ \mu g$/ovary in the freshly emerged unfed female mosquitoes. The level of proteins increased dramatically four times after first gonotrophic cycle ($6.2 \pm 0.4\ \mu g$) on day-9 compared to freshly

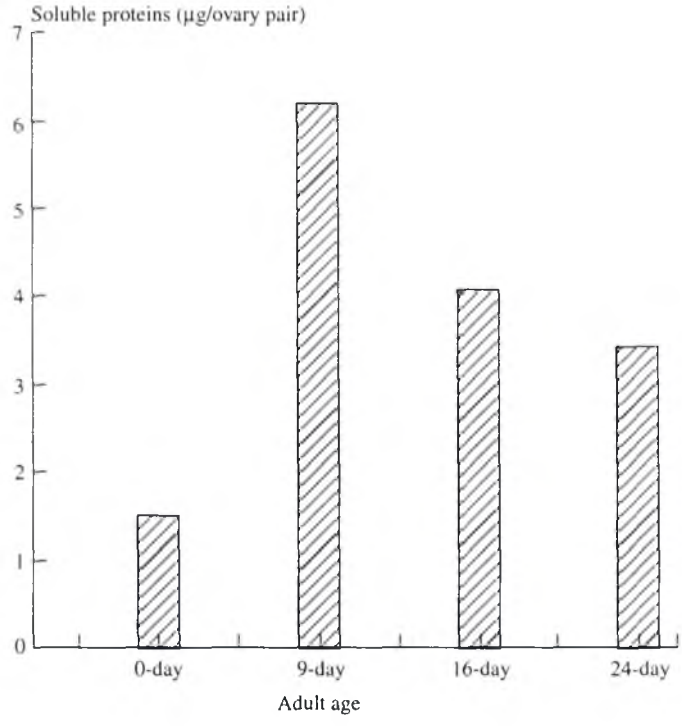

FIGURE 2. Variation in protein contents in the ovaries during development and ageing of *A. stephensi*.

emerged females. After second gonotrophic cycle, this level decreased by about 35% ($P < 0.01$) per ovary pair as compared to ovaries of young females. Its level was reduced further by 15% ($P < 0.05$) per ovary pair in aged mosquitoes (Fig. 2).

Polypeptides during sugar feeding

In total, 21 polypeptides disappeared from the ovaries when female mosquitoes were maintained exclusively on sugar/glucose diet as compared to control females of their corresponding age. Three polypeptides (59, 119 and 185 kDa) generally disappeared from the ovaries of all the stages. In addition, 7 polypeptides (21, 70, 104, 167, 173, 178 and 180 kDa) on day-9; 13 polypeptides (10.5, 11.5, 26, 29, 42, 104, 116, 134, 140, 143, 167, 173 and 178 kDa) on day-15 and 3 polypeptides (26, 37 and 116 kDa) on day-24 also disappeared in glucose fed females. However, these were present during haematophagy (Fig. 1). Only 2 polypeptides i.e. 38.5 kDa on day-9 and 18 kDa on day-24 old mosquitoes were induced in the ovaries upon sugar feeding.

DISCUSSION

The haemoglobin content of the blood meal provides the major protein source for egg development (Briegel, 1985). This could be the reason for the disappearance of 21 polypeptides in the ovaries of sugar fed females. These polypeptides may be essential for the ovary maturation and differentiation. However, the 4-fold increase in protein content in the ovaries during first gonotrophic cycle could be ascribed to the synthesis of 6 polypeptides (58, 70, 91, 95, 109 and 116 kDa) on day-4 and 4 polypeptides (37, 100, 140 and 143 kDa) on day-9. It was also well coincidental that during the same period, 8 polypeptides (24, 59, 62, 70, 118, 121, 140 and 150 kDa) on day-4 and 2 polypeptides (46 and 73 kDa) on day-9 also disappeared from the haemolymph (Gakhar and Shandilya, 2000). These proteins could selectively be taken up by developing oocytes and may be termed as vitellogenins. The actual titer of vitellogenins in the haemolymph are controlled by the uptake of vitellogenins by the ovary in *Ae. aegypti* (Ma *et al.*, 1986). Vitellogenins synthesized by the fat body reach maximal level by 30 hour (Hogg *et al.*, 1997), who have also shown that the timing of protein accumulation in *A. stephensi* is similar to that in *Ae. aegypti* (Redfern, 1982; Yonge and Hagedorn, 1977).

The fate of blood meal protein in both aedine and anopheline species has been studied by Briegel (1990). Amino acids from protein hydrolysis are incorporated directly into vitellogenins, deaminated to form carbohydrates and lipids for yolk, incorporated into body reserves or degraded (Briegel, 1990). The relative proportion of blood meal energy values diverted to these functions varies depending upon the species, female size, blood meal size and host species. In anophelines, up to 30% of blood meal proteins are converted into yolk protein and lipids, upto 15% are incorporated into extra-ovarian material, and the remaining 55% are degraded (Briegel and Rezzouico, 1985). Thus, when large blood meals are taken, protein is far in excess of that utilized for eggs production.

The decline in protein content (by about 35%) after third gonotrophic cycle could partly be ascribed to the disappearance of 6 polypeptides (11.5, 18, 29, 30, 137 and 173 kDa) after the process of egg laying as also reported in other mosquito, *A. albimanus* (Brogdon, 1984) and partly to the degradative processes associated with senescence. One of the theories of ageing is that the reduced protein synthesis is a feature of ageing process. A decline in protein content during adult life is also correlated to senescence in *A. stephensi* (Gakhar and Singh, 1998). Characterization of both induced and repressed proteins may help understand the fine regulation of vitellogenins in mosquitoes. Analysis of these regulated proteins may shed light on signals controlling vitellogenesis.

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External genitalia and morphometry of male and female reproductive systems of *Samia cynthia ricini* Boisduval (Lepidoptera: Saturniidae)

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ABSTRACT: The structure of reproductive systems in eri silk moth resembles to that of any lepidopterans. However, in the male, the vas deferens is narrow at its distal half, ejaculatory duct becoming narrow for a short length before ending in aedeagus and aedeagus being narrow at the base and broader at the tip with vesica at its end. Similarly, in female, the reproductive system with a triangular yellowish body at the tip of ovarioles and the bursa copulatrix having bisac are the distinguishing features.

KEYWORDS: External genitalia, reproductive system, *Samia cynthia ricini*

INTRODUCTION

The silk produced by *Samia cynthia ricini* Boisduval (Lepidoptera: Saturniidae) stands second among non-mulberry silks produced in India (Anonymous, 1999). Although information on host plants, rearing of eri silkworms and marketing of cocoons (Sarkar, 1988) and detailed embryological development has been gathered (Krishnappa, 1989; Reddy *et al.*, 2000), yet, literature pertaining to anatomical studies of larval, pupal and adult stages is scanty. In tasar silkworm, the anatomical features of most prominent systems in larval and adult stages have been documented (Jolly *et al.*, 1979). Kumar (1990) has studied the morphology of reproductive systems in *Antheraea mylitta* Drury. The productivity of silk is directly related to the reproductive potential of the sericigenous insects. Therefore, a study was made to know the morphology of external genitalia as well as the reproductive systems in both male and female moths of *S. c. ricini*.

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MATERIAL AND METHODS

The moths required for the present study were obtained from the culture raised on castor leaves. To study the external genitalia, abdomen of both the male and female moths was detached and placed in 10 percent potassium hydroxide solution overnight. These were repeatedly washed in distilled water to remove the digested soft tissues by pressing the abdomen with a pair of bent needles. The cleared specimens were transferred to glycerin in a glass cavity dish and dissected under stereoscopic microscope to expose the genitalia. The terminology of Snodgrass (1935) was adopted to describe the parts of the genitalia.

To study the internal morphology of reproductive system, moths were anaesthetized after 10 minutes of eclosion; appendages were removed and transferred to normal pH saline in a dissecting tray. The abdomen was cut open and reproductive organs were exposed. The organs were separated from the moth and measurements were made under binocular stereoscopic microscope. Thirty moths of each sex were used in the present study. The bulkiness of female reproductive system was determined by water displacement method using 10 moths and calculated as the ratio of volume of female reproductive system to the volume of entire female moth.

RESULTS AND DISCUSSION

External genitalia of male

The male external genitalia, modified from 8 and 9th abdominal segments, is located towards the posterior of conspicuous 7th abdominal segment. It consists of dorsal plate, the 'tegumen' [Fig. 1(a)]. The latter at its hind margin carries a lobe like process, the 'uncus'. On the surface of tegumen orienting towards dorso-lateral side are a pair of spiny-hook-like structures termed 'accessory harpes' [Fig. 1(b)]. The ventral aspect comprises 'vinculum' and it is furnished with a pair of spine like 'harpes' arising from its lateral directions. Aedeagus emerges out between the harpes [Fig. 1(c)]. Accessory harpes have been reported to be conspicuously present in the males of *A. mylitta*. However, their location has been found to be on either side of vinculum, ventrally (Sen and Jolly, 1971).

Reproductive system of male

The structure of male reproductive system in *S. c. ricini* [Fig. 1(d)] is similar to that of other lepidopterans. A pair of light greenish-yellow testes is ventro-laterlly located in the 5th abdominal segment. Each testis is 0.331 cm × 0.214 cm and has four testicular follicles as seen in most of the lepidopterans (Richards and Davies, 1993). In contrast the testis in *A. mylitta*, has been reported to be broader than its length (Kumar, 1990). The testicular follicles unite to form vas efferens, which in turn lead to vas deferens. Vas deferens arises from the base of each testis and unites at a bulbus structure called vesicula seminalis. As evident from measurements given in Table 1, each vas deferens is broader at proximity to testis and is narrower towards the seminal vesicle, which again broadens before uniting with vesicula seminalis. Such narrowing of vas deferens

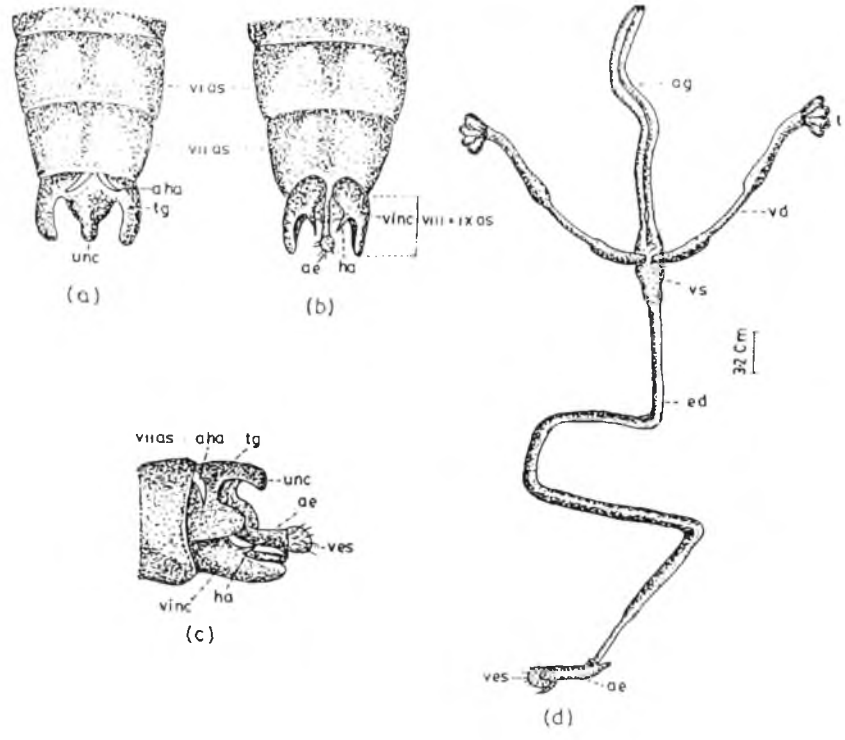

FIGURE 1. External genitalia [dorsal (a), ventral (b) and lateral (c) views] and reproductive system (d) in male *Samia cynthia ricini* Boisduval (as - Abdominal segment, ag - Accessory glands, ae - Aedeagus, ed - Ejaculatory duct, ha - Harpes, aha - Accessory harpes, vd - vas deferentia, vs - Vesicula seminalis, t - testis, tg - Tegumen, Vinc - Vinculum, unc - Uncus, ves - Vesica).

is not seen in other silk moths *viz.*, *B. mori* (Tazima, 1978) and *A. mylitta* (Jolly *et al.*, 1979; Kumar, 1990). Vas deferentia lead to or open into barrel shaped vesicula seminalis (0.65 cm long and 0.208 cm at its widest portion). Towards its anterior end it receives a pair of closely appressed whitish accessory glands measuring 1.74 cm in length and 0.1 cm in width. From the posterior end of seminal vesicle, arises 5.171 cm long ejaculatory duct. Anterior portion of the ejaculatory duct (4.74 cm) is broader (0.111 cm) and distal portion (0.431 cm) is narrow (0.064 cm). The narrow end of ejaculatory duct is specific to *S. c. ricini* and is not seen in *B. mori* (Tazima, 1978) and *A. mylitta* (Jolly *et al.*, 1979; Kumar, 1990). The ejaculatory duct terminates into aedeagus, which is 0.433 cm long brownish structure. It has narrow base (0.104 cm) and broader tip (0.171 cm) ending in yellowish lobe like 'vesica', similar to the one seen in lepidopterans (Viette, 1948). This feature is again peculiar to *S. c. ricini*.

External genitalia of female

The female genitalia is located on ventral side of 8 and 9th segments with modified terminal 10th segment functioning as retractile ovipositor. Towards the posterior

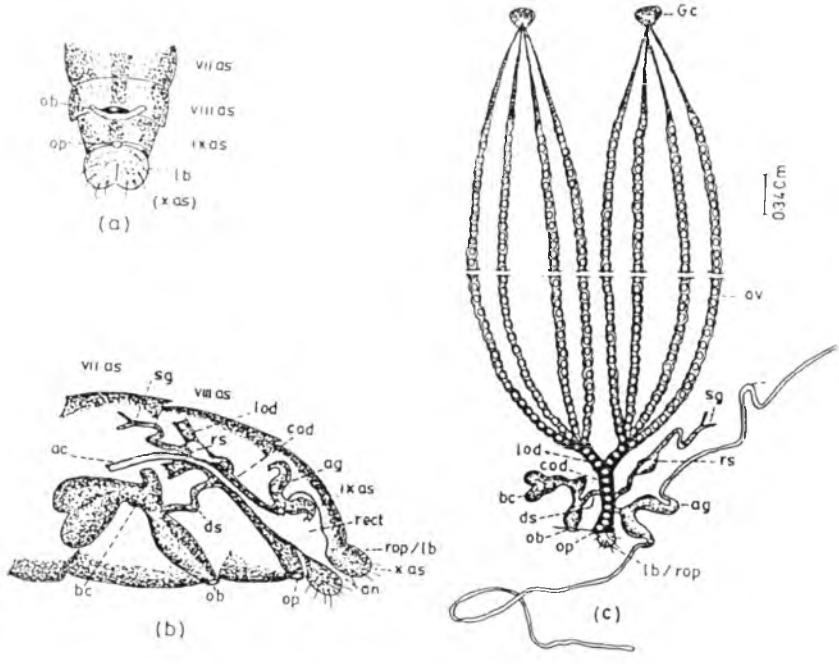

FIGURE 2. External genitalia (a), longitudinal sectional view of terminal segments (b) and reproductive system in female *Samia cynthia ricini* Boisduval (as - Abdominal segment, ag - Accessory glands, ac - Alimentary canal, an - Anus, bc - Bursa copulatrix, cod - Common oviduct, ds - ductus seminalis, Gc - Gonadal case, lb - Labin, lod - Lateral oviduct, ob - Ostium bursae, op - Ovipore, ov - Ovary with four ovarioles, rect - Rectum, rs - receptaculum seminalis, rop - Retractile ovipositor, sg - spermathecal gland).

margin of 8th segment, ostium bursae is located with a sternite bracing it posteriorly [Fig. 2(a)]. The ovipore is present on the posterior margin of 9th abdominal segment in close proximity to retractile ovipositor [Fig. 2(b)]. In lepidopterans (Richards and Davies, 1993), including *B. mori* (Tazima, 1978) and *A. mylitta* (Sen and Jolly, 1971), the terminal segment being attenuated and telescoped so as to function as retractile ovipositor, has been well documented.

Reproductive system of female

A pair of ovaries almost occupied the entire abdominal cavity. Each ovary consists of four polytrophic ovarioles measuring 11.493 cm in length (Table 2). The proximal tip of all the ovarioles are united and held together by a yellowish triangular structure that is not known to be present in other lepidopterans (Sen and Jolly, 1971; Tazima, 1978; Richards and Davies, 1993). This structure may be the larval gonadal case, which still persists after morphogenesis. The location of the ovariole tip is not specific to any segment but is located towards dorsal side. The ovarioles of each side unite to form a lateral oviduct measuring 0.351 cm in length and 0.101 cm in width (Table 2). The

TABLE 1. Morphometry of male reproductive system of *S. c. ricini* (Each observation is a mean of 30 individuals)

Organ	Length (cm)	Width (cm)
Testis	0.331 ± 0.046	0.214 ± 0.034
Vas deferens		
Broad tube	0.760 ± 0.055	0.113 ± 0.024
Narrow tube	0.760 ± 0.048	0.040 ± 0.007
Seminal vesicle	0.650 ± 0.050	0.208 ± 0.022
Ejaculatory duct		
Broad tube	4.74 ± 0.472	0.111 ± 0.023
Narrow tube	0.431 ± 0.049	0.064 ± 0.005
Total length	5.171 ± 0.481	—
Aedeagus		
Base	—	0.104 ± 0.014
Tip	—	0.171 ± 0.027
Total length	0.433 ± 0.043	—
Accessory gland	1.740 ± 0.117	0.100 ± 0.010

two lateral oviducts unite to form the common oviduct that leads to ovipore on the ventral side of 9th abdominal segment at the base of the ovipositor [Fig. 2(b)]. The common oviduct measured 0.541 cm in length (Table 2) and narrow at the proximal end (0.101 cm in width) and broader at distal end close to the ovipositor (0.209 cm in width).

The bursa copulatrix is present towards the ventro-anterior aspect of common oviduct and is bisaced [Fig. 2(b) and (c)], which is unique to *S. c. ricini*. It measured 0.539 cm in length from the proximal tip to distal end. It ends into ostium bursae. The width of the proximal and distal sac was 0.279 and 0.215 cm, respectively. The bursa copulatrix is connected to the common oviduct on its ventral aspect by a seminal duct, which measured 0.196 cm in length and 0.035 cm in width (Table 2). The spermatheca that opens into the common oviduct at its dorso-posterior aspect, consists of spermathecal sac (receptaculum seminalis) and gland. The spremathecal sac is 0.198 cm in length while the gland of spermatheca is bifid at its tip and measured 1.12 cm in length. A pair of accessory glands, consisting of bulbous sigmoid shaped greenish basal structure with long and blunt tube, opens into the common oviduct just below the spermatheca [Fig. 2(b) and (c)]. The basal structure measured 0.708 cm in length and 0.316 cm in width, while the tube was 9.905 cm in length and 0.062 cm in width (Table 2).

Retractile ovipositor (Labin)

It is found at the tip of the abdomen in close association to reproductive system. Its association with reproductive system is only to sense the substratum during the act

TABLE 2. Morphometry of female reproductive system of *S. c. ricini* (Each observation is a mean of 30 individuals)

Organ	Length (cm)	Width (cm)
Ovariole	11.493 ± 1.369	—
Lateral oviduct	0.351 ± 0.091	0.101 ± 0.011
Common oviduct	0.541 ± 0.049	—
Distal end	—	0.209 ± 0.002
Proximal end	—	0.101 ± 0.008
Lateral oviduct	0.351 ± 0.091	0.101 ± 0.011
Bursa copulatrix	0.539 ± 0.047	
Proximal sac	—	0.279 ± 0.023
Middle	—	0.099 ± 0.009
Distal sac	—	0.215 ± 0.025
Seminal duct	0.196 ± 0.013	0.035 ± 0.004
Spermatheca		
Spermathecal sac	0.198 ± 0.015	—
Spermathecal gland	1.12 ± 0.091	—
Accessory gland		
Base	0.708 ± 0.026	0.316 ± 0.032
Tube	9.905 ± 0.674	0.062 ± 0.006
Labin (retractile ovipositor)	0.102 ± 0.009	0.154 ± 0.014
Bulkiness ratio*	0.5684 ± 0.0409	

* Mean of 10 female moths

of oviposition as in case of other lepidopterans (Sen and Jolly, 1971; Richards and Davies, 1993). It measured 0.102 cm in length and 0.154 cm in width (Table 2).

The bulkiness ratio of female reproductive system to the body in terms of volume was 0.5684, indicating nearly 57 percent of the space being occupied by the reproductive system. No relevant literature on this aspect has been documented.

It is obvious from the present results that the male reproductive system in eri moth is unique compared to other sericigenous insects with reference to vas deferens being narrow at its distal half, ejaculatory duct becoming narrow for a short length before ending in aedeagus and aedeagus being narrow at the base and broader at the tip with vesica at its end. Similarly, in female the reproductive system with a triangular yellowish body at the tip of ovarioles, which probably is reminiscent of larval gonadal case and the bursa copulatrix having bisac are the distinguishing features.

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Effect of cytoplasmic polyhedrosis on amylase and succinate dehydrogenase activity levels in silkworm *Bombyx mori* L.

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ABSTRACT: Two races of mulberry silkworm namely Pure Mysore and NB_4D_2, at fifth instar first day were inoculated with two different doses of cytoplasmic polyhedral inclusion bodies *viz.*, 1.562×10^6/ml and 3.125×10^6/ml by oral injection. The effects of cytoplasmic polyhedrosis on the amylase and succinate dehydrogenase activity levels in haemolymph as well as midgut tissue were studied. In the diseased larvae, the enzyme activity levels were gradually decreased as the disease progressed. Such changes in the infected larvae, depicts the possible defense cellular adjustment of the host in response to pathogen attack.

KEYWORDS: *Bombyx mori*, polyhedrosis, amylase, succinate dehydrogenase

INTRODUCTION

In the tissues of insects with an infectious disease, various biochemical, physiological and cytomorphological alterations can be observed (Martignoni, 1964; Sujak *et al.*, 1978). Most of the biochemical studies associated with cytoplasmic polyhedrosis are limited to daily changes in the quantity of nucleic acid and protein in the blood and midgut (Kawase and Hayashi, 1965); quantitative and qualitative changed in haemolymph protein (Mahesha *et al.*, 2000); protein metabolism in the midgut epithelium (Watanabe, 1970); changes in the amino acids of haemolymph and midgut epithelium (Kawase, 1965) and activities of several carboxylases (Yaginuma *et al.*, 1990). The analysis of enzymes like amylase, succinate dehydrogenase (Mahesha, 1997), alkaline phospatase and alkaline protease (Lakshmi Kumari, 1995) may help

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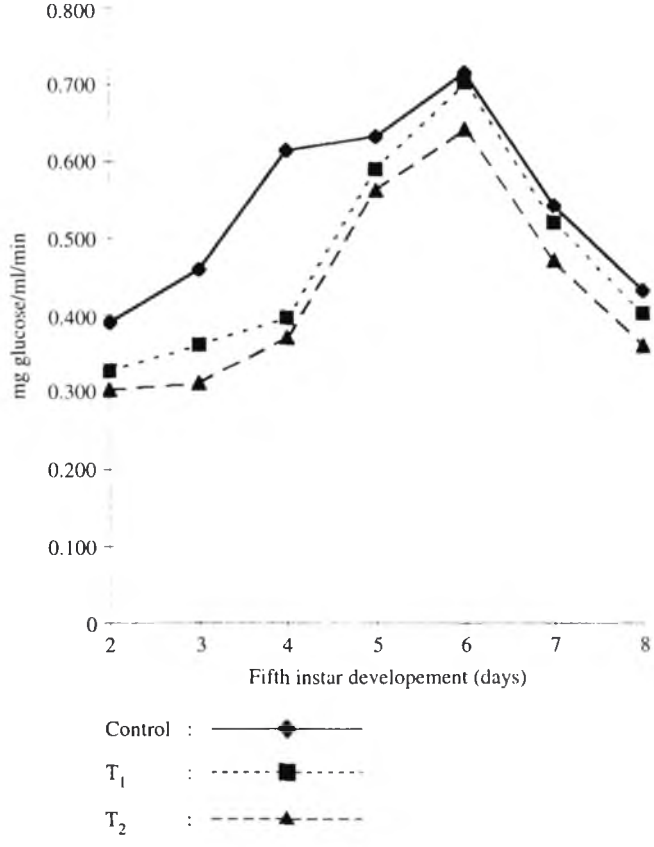

FIGURE 1. Amylase activity levels in haemolymph of Pure Mysore larvae during cytoplasmic polyhedrosis.

in the silkworm breeding programme for cocoon characters and disease resistance. However, studies combining digestive and oxidizing enzymes with cytoplasmic polyhedrosis are rather scarce. Hence, the present investigation was undertaken to study the amylase and succinate dehydrogenase activity levels in the haemolymph and midgut tissue, during the course of cytoplasmic polyhedrosis.

MATERIALS AND METHODS

Two mulberry silkworm races namely Pure Mysore (multivoltine) and NB_4D_2 (bivoltine) at the age of fifth instar first day and stocks of cytoplasmic polyhedrosis virus (*Bm*CPV) were used for the study.

The cytoplasmic polyhedral inclusion bodies (PIBs), obtained from Sericulture Department, University of Agricultural Sciences, Bangalore, India, were *per orally* inoculated into the silkworm larvae for multiplication. Isolation and purification of

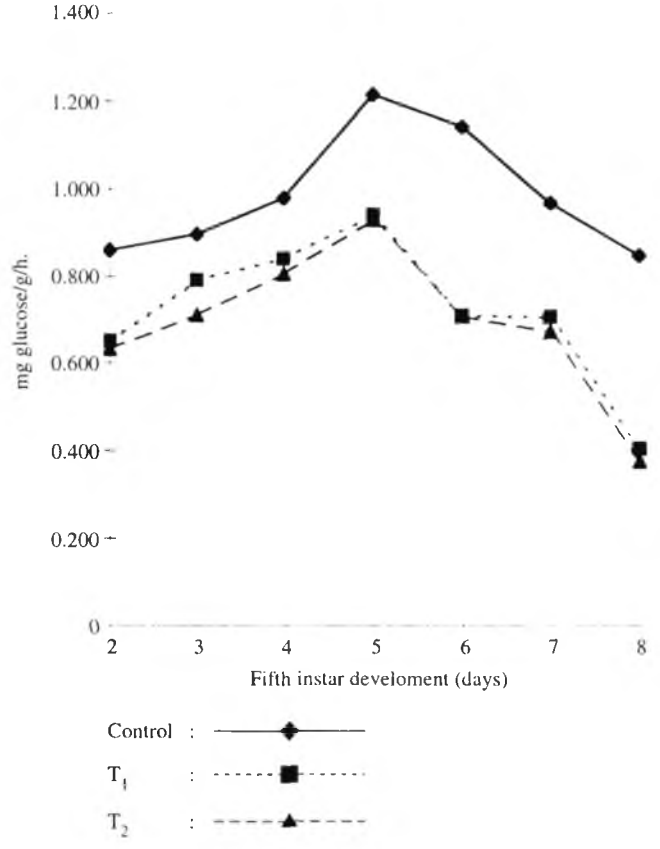

FIGURE 2. Amylase activity levels in midgut of Pure Mysore larvae during cytoplasmic polyhedrosis.

PIBs were carried out by following the method described by Balakrishnappa and Honnaiah (1992). From the stock suspension, two doses *viz.*, 1.562×10^6/ml (T_1) and 3.125×10^6/ml (T_2) were prepared.

The silkworm rearing was conducted in the laboratory following the method described by Krishnaswami (1978). 40 μl of final concentration of PIBs suspension prepared in 0.75% NaCl solution was administered separately to each worm into the gut by 'oral injection'. The control worms received the same amount of NaCl solution only. For each concentration, 60 worms in triplicate were taken. After inoculation, the larvae were allowed to continue further development.

About 5–10 larvae were collected daily at regular interval of 24 h from the time of inoculation from each batch until the onset of spinning. The abdominal legs were punctured and the haemolymph was collected in pre-chilled eppendrof tubes containing 1 mM thiourea crystals to prevent oxidation. The midgut tissue

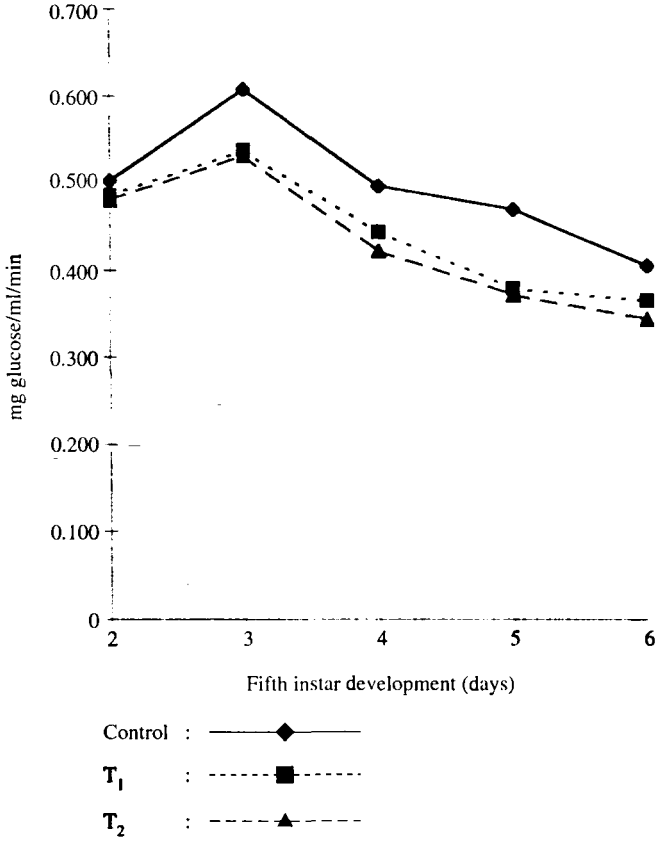

FIGURE 3. Amylase activity levels in haemolymph NB_4D_2 larvae during cytoplasmic polyhedrosis.

was obtained from five larvae by dissecting in ice cold water and the gut contents were removed. The tissue was thoroughly washed in distilled water. A 1% (w/v) homogenate of the midgut tissue was prepared in pre-cooled distilled water using mortar and pestle. Both haemolymph and midgut tissue homogenate samples were centrifuged at 3000 rpm for 10 minutes. The clear supernatant was used for the assay of amylase and succinate dehydrogenase activity.

Quantitative analysis of amylase activity was done in haemolymph and midgut tissues following the method of Ishaaya and Swirski (1976). The values were expressed as mg glucose released/ml/min and mg glucose released/g/min for haemolymph and midgut tissue respectively. Succinate dehydrogenase activity levels were estimated by the method of Nachlas *et al.* (1960). The values were expressed as μmoles farmazan formed/ml/h and μmoles farmazan formed/g/h for haemolymph and midgut tissue respectively.

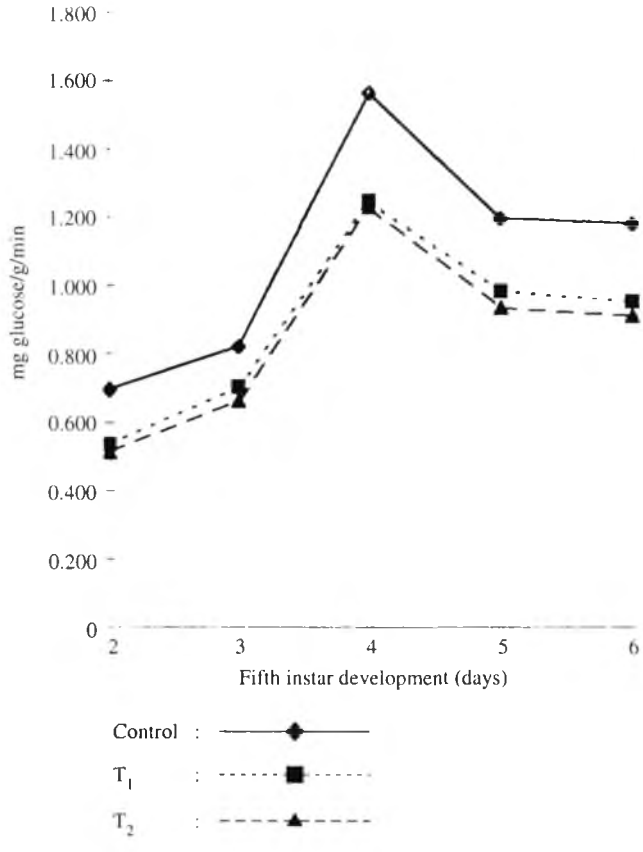

FIGURE 4. Amylase activity levels in midgut of NB_4D_2 larvae during cytoplasmic polyhedrosis.

RESULTS AND DISCUSSION

The amylase activity levels in haemolymph of control worms of Pure Mysore race was increased with the increase in the age during fifth instar and reached its peak on the sixth day. However, a gradual reduction in the activity was observed on the seventh and eighth day. The *Bm*CPV inoculated silkworms of both T_1 and T_2 sets also showed the same pattern of enzyme activity as that of control silkworms, but a reduced rate of enzyme activity was observed (Fig. 1). The average enzyme activity during fifth instar exhibited by control set was 0.54 mg/ml/min, followed by T_1 (0.47 mg/ml/min) and by the T_2 (0.43 mg/ml/min) sets. In case of midgut tissue of the control worms, the amylase activity levels showed gradual increment from second day to fifth day; but thereafter showed gradual reduction in its activity until the end of the last instar. The *Bm*CPV inoculated batches also followed the same pattern of enzyme activity as in the case of the control worms but with reduced

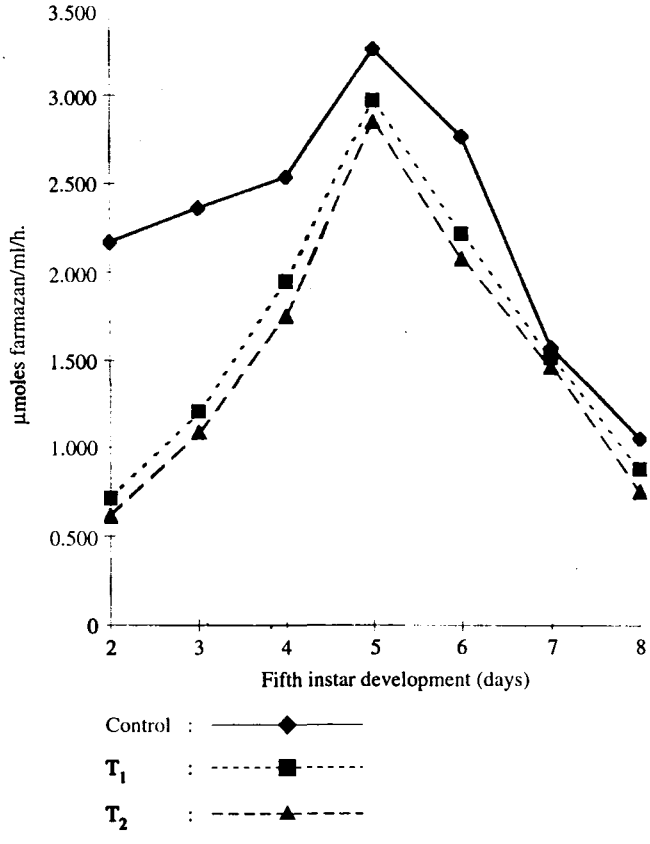

FIGURE 5. Succinate dehydrogenase activity levels in haemolymph of Pure Mysore larvae during cytoplasmic polyhedrosis.

rate of enzyme activity (Fig. 2). The average amylase activity was high in control (0.99 mg/g/min) followed by T_1 (0.72 mg/g/min) and T_2 (0.72 mg/g/min) sets. In the case of NB_4D_2 larvae, the amylase activity levels in the haemolymph of control silkworms showed their peak activity on the third day of fifth instar. From fourth day onwards, a gradual reduction was noticed. The *Bm*CPV inoculated batches, also showed similar pattern with reduced rate of enzyme activity (Fig. 3). A higher amylase activity was observed in control worms (0.49 mg/g/min) followed by T_1 (0.44 mg/g/min) and T_2 (0.43 mg/g/min) sets. In the midgut tissue of control worms, the amylase activity showed its peak activity levels on the fourth day and thereafter showed gradual reduction till the end of fifth instar. In case of diseased worms, the pattern of enzyme activity was similar, but with reduced rate of enzyme activity (Fig. 4). The amylase activity exhibited by control worms was more (1.09 mg/g/min) followed by T_1 (0.88 mg/g/min) and T_2 (0.85 mg/g/min) batches.

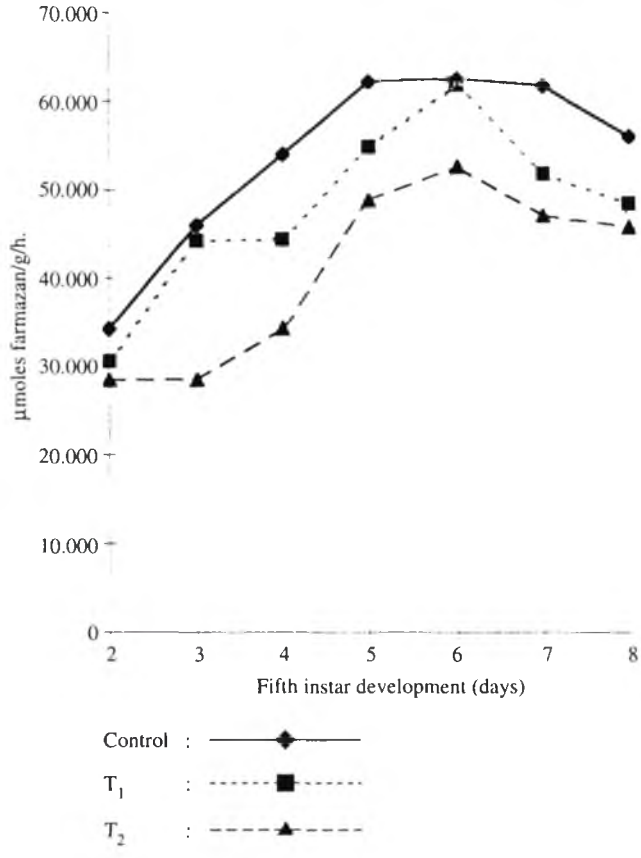

FIGURE 6. Succinate dehydrogenase activity levels in midgut of Pure Mysore larvae during cytoplasmic polyhedrosis.

The succinate dehydrogenase activity in the haemolymph of control larvae of Pure Mysore race showed a gradual increment from second day to fifth day. Again, from sixth day, it showed gradual decrease until the end of fifth instar. The *Bm*CPV inoculated batches of both T_1 and T_2 also showed the same pattern with reduced rate of enzyme activity (Fig. 5). The succinate dehydrogenase activity exhibited by control worms was 2.24 μmoles/ml/h followed by T_1 set with 1.63 μmoles/ml/h and T_2 set with 1.51 μmoles/ml/h. In the midgut tissue, the succinate dehydrogenase showed gradual increment from second day to sixth day; but thereafter showed gradual reduction in its activity till the end of fifth instar. In case of *Bm*CPV inoculated batches, the pattern of enzyme activity was similar apart from the reduced rate (Fig. 6). A higher activity was observed in control worms (53.84 μmoles/g/min) followed by T_1 (47.97 μmoles/g/h) and T_2 (40.78 μmoles/g/h) sets. The succinate dehydrogenase activity levels in the haemolymph of control larvae of NB_4D_2 showed

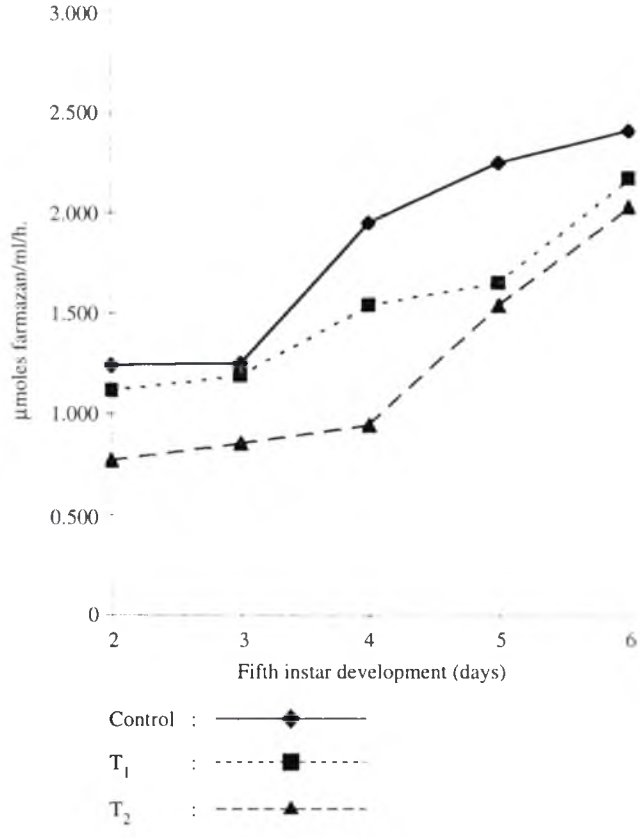

FIGURE 7. Succinate dehydrogenase activity levels in haemolymph of NB_4D_2 larvae during cytoplasmic polyhedrosis.

its maximum enzyme activity on sixth day of fifth instar. There was gradual increase in the enzyme activity from first to sixth day. The batches of both T_1 and T_2 also followed the same pattern but with reduced rate of enzyme activity (Fig. 7). The enzyme activity exhibited by control worms was more (1.82 μmoles/ml/h) followed by T_1 (1.53 μmoles/ml/h) and T_2 (1.23 μmoles/ml/h) batches. In the midgut tissue, the succinate dehydrogenase exhibited its peak activity on the sixth day in the control as well as *Bm*CPV inoculated sets. However, the diseased silkworms exhibited a reduced rate of enzyme activity (Fig. 8). This reduction was directly proportional to the quantity of inoculum. A higher enzyme activity was observed in control worms (59.19 μmoles/g/min) followed by T_1 (52.06 μmoles/g/h) and T_2 (46.84 μmoles/g/h) sets.

The CPV infection showed significant reduction in amylase and succinate dehydrogenase activity in both Pure Mysore and NB_4D_2 races. The reduced rate of enzyme activity in haemolymph and midgut tissue might be due to either of the following possi-

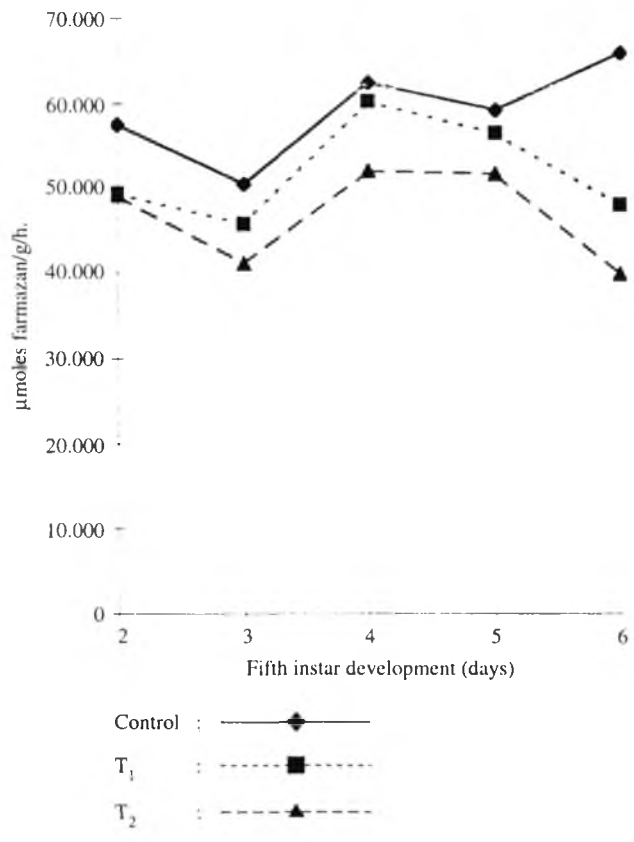

FIGURE 8. Succinate dehydrogenase activity levels in midgut of NB_4D_2 larvae during cytoplasmic polyhedrosis.

bilities. Firstly, the degradation of silkworm proteins and utilization of amino acids by virus for the synthesis of polyhedral proteins. This hypothesis is supported by Watanabe and Kobayashi's (1969) work. They suggested that the synthetic activity of silk protein in the silk gland cells of *B. mori* is greatly lowered by infection with nuclear polyhedrosis virus (NPV), and on the other hand, the synthesis of polyhedron protein becomes active as the polyhedrosis progresses. Ramaiah Veerabasappa Gowda (1970), and Veerabasappa Gowda and Ramaiah (1976) found increased transaminases activity in the haemolymph of silkworm larvae during the course of nuclear polyhedrosis. The increased concentration of these enzymes might suggest increased protein degradative activity in the host, thus facilitating the production of viral proteins and the multiplication of polyhedral viral particles. In addition, Watanabe (1970) found enhanced protein metabolism in the midgut epithelium of *B. mori* infected with *Bm*CPV. Kawase (1965), also observed that most of the amino acids decreased in the haemolymph and midgut epithelium of *Bm*CPV infected larvae. Their amount ranged from one-half to one-tenth

of the normal amount. Yaginuma *et al.* (1990) reported that the energy source needed for multiplication of *Bm*CPV are provided from trehalase in haemolymph through a mediation of trehalase during middle stage of infection and from glycogen in the cells through phosphorylase during the last stage. The results from all these authors confirm that the energy source needed for multiplication of *Bm*CPV is provided from the degradation of the haemolymph and midgut tissue proteins. Secondly, since the cytoplasmic polyhedrosis virus destroys the midgut tissue of the digestive system, it cannot perform its normal function *viz.*, digestion and absorption. The *Bm*CPV inoculated silkworms showed less protein content with altered protein fractions (Mahesha *et al.*, 2000), low level of blood glucose, reduced rate of enzyme activity reflects the utilization of less food material, reduced rate of conversion and metabolism resulting in the less production in the surviving silkworms (Mahesha, 1997). Such a basic knowledge about these biochemical aspects during polyhedrosis might offer an important tool to evolve disease resistant breeds.

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Relative susceptibility of different life stages of *Plutella xylostella* (L.) to entomopathogenic nematode, *Heterorhabditis bacteriophora* Poinar

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ABSTRACT: Efficacy of *Heterorhabditis bacteriophora* Poinar, an entomopathogenic nematode was tested against various developmental stages of *Plutella xylostella* (L.). The laboratory bioassay revealed that no nematode infection was observed in the egg masses. The final instar larvae were most susceptible amongst all test stages of the insect with a LC_{50} value of 9.16 IJs/insect. The prepupal stage was equally susceptible with LC_{50} value of 10.13 IJs/insect. However, LC_{50} values recorded for freshly formed pupae and cocoons were 27.1 and 28.17 IJs/insect, respectively. Two day old pupae (cocoons) proved to be most resistant with a LC_{50} value of 86.88 IJs/insect.

KEYWORDS: Relative-susceptibility, entomopathogenic nematode, *P. xylostella*, *Heterorhabditis bacteriophora*

Diamond back moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is a widely distributed pest of crucifers like cabbage and cauliflower in many tropical and temperate regions throughout the world (Talekar, 1992; Talekar and Shelton, 1993) in general and India in particular (Srinivasan, 1988; Rai *et al.*, 1992). The management of this pest is becoming a problem, as it is fast developing insect and also due to its speedily developing multiple resistance to insecticides. In recent past, Shinde and Singh (2000) reported *Heterorhabditis bacteriophora*, amongst the eight test entomogenous nematodes, as an effective tool for the management of DBM. In the present studies, the bioefficacy of *H. bacteriophora* vis-á-vis different developmental stages of DBM has been extensively investigated, so that an effective field dose of pathogenic nematode may be developed for the control of DBM under field conditions.

The different developmental stages of DBM maintained on cabbage seedlings were exposed to different treatments of *H. bacteriophora*. The different stages used were: eggs (1–24 h old), final instar larvae (1–24 h old), the prepupal stage (final instar larvae stopped feeding and initiated pupal formation), cocoon (pupa which has just secreted the cocoon), pupa without cocoon (0–24 h cocoon whose covering mechanically

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removed), pupa (2 day old pupa with hard cocoon), and adult (one day old). The test nematode, *H. bacteriophora* was multiplied *in vivo* on greater wax moth (GWM), *Galleria mellonella*, larvae at 25 °C, using the method of Dutky *et al.* (1964). The infective juveniles (IJs) of the pathogenic nematode were stored in tissue culture flask (100 ml capacity) at 10 °C.

Bioefficacy tests

Egg-stage

The cabbage leaves with laid eggs on them were clipped off from the seedling and counted number were placed over a filter paper in the arena circle, (15 mm in diameter) pre-impregnated with IJs suspension (500 IJs/ml). The number of eggs hatched and reaching upto second instar stage were recorded. The clipped off egg masses were sprayed with higher dose of 5000 IJs/ml and motality count recorded.

Different developmental stages

The larvae, prepupae and pupae were individually exposed to IJs in arena circle. The arena was prepared by fixing a 5 mm thick PVC ring (inner diameter 15 mm) at the centre of a glass slide (75 mm × 25 mm). The arena was closed with a 20 mm× 20 mm glass coverslip. A thin coat of grease was applied over the top surface of the ring for holding the coverslip. The IJs suspended in 0.1 percent formalin solution were evenly distributed over the filter paper lying at the base of each arena. The test doses were 5, 10, 20, 30 and 40 IJs/arena/insect. Different test stages of the insect were then placed in the arena (one insect/arena). In each treatment dose 30 insects of a developmental stage were exposed. Parallel checks were run with each treatments using 0.1 percent formalin solution only. Mortality counts were recorded 48 h after exposure. The data so obtained were subjected to probit analysis (Finney, 1952) for determining the LC_{50}.

Adult stage

The filter paper impregnated with IJs/ml was placed at the bottom of 100 ml glass beaker. Thereafter, twenty moths were released in the beaker which was then covered with a muslin cloth and fastened with a rubber band. Mortality counts were made at 24, 48 and 72 h post exposure. The dead moths were kept on nematode emergence traps. Emergence of the nematodes from the carcas confirmed the mortality of moths due to nematodes. Ten dead insects of different developmental stages obtained from bioassay tests were used for determining the IJs production density. Counting of IJs from each cadaver was made as per the procedure of Shinde (2000).

Investigations revealed no nematode infection in any of the egg masses, even after spraying them with higher concentration of IJs suspension (5000 IJs/ml). The hatching in eggs treated with different treatment doses of nematode and in the untreated control; no marked difference in the egg hatchability (80–85%) was observed.

The final instar larvae were most susceptible amongst the all test stages of the insect. The least LC_{50} value of 9.15 IJs/insect was recorded for them. The prepupal

TABLE 1. Susceptibility of different life stages of *P. xylostella* to *Heterorhabditis bacteriophora* and its propagation in various development stages of DBM

Development stage	Dose mortality relationship (regression equation)	LC_{50} (IJs/insect)	Relative Susceptibility	Mean No. of IJs/mg body weight
Final instar larvae	$Y = 1.9682976 + 3.1510331x$	9.16	3.064	271.42
Prepupa	$Y = 2.2636807 + 2.721139x$	10.13	2.771	240.85
Freshly formed pupa without cocoon	$Y + 2.1663394 + 1.9771695x$	27.11	1.035	115.63
Freshly formed pupa with cocoon	$Y = 1.9917904 + 2.077120x$	28.07	1.000	107.46
Two day old pupa without cocoon	$Y = -0.5098779 + 2.8432156x$	86.08	0.324	23.41
Adult	–	–	–	356.45

stages were equally sensitive, here, the LC_{50} value was 10.13 IJs/insect. However, freshly formed pupae and cocoon gave the LC_{50} value of 27.1 and 28.17 IJs/insect, respectively. 2 day old pupae were the most resistant among the test developmental stages giving the LC_{50} value of 86.68 IJs/insect (Table 1). When moths were exposed to IJs on filter paper, percent mortality at 24, 48 and 72 h after release was 20, 90 and 100 percent, respectively. Propagation and emergence of nematodes from the cadavers were observed in all larval and prepupal stages. However, in freshly formed pupae (with or without cocoon) the nematodes did not emerge themselves in 20 to 30 percent cadavers, but were recovered after dissecting the host body.

Scanty information is available on the pathogenecity of entomopathogenic nematodes on the different developmental stages of phytophagous insects in general and DBM in particular. Kondo (1989) observed that prepupal stage of *Spodoptera litura* was more susceptible than to the larval stages to *S. feltiae*. Morris (1985) reported 66.6% mortality of DBM pupae by *H. bacteriophora* and *S. feltiae* at a dose of 125 IJs/pupa. Contrary to the findings of Kondo (1989), it was observed that the larval stage was most susceptible to *H. bacteriophora* followed by pre-pupal stage of DBM.

In the present study, the laboratory bioassay revealed that final instar larvae were 3.06 times relatively more susceptible than the freshly formed pupae with cocoon. The grading of relative susceptibility decreased with the further development of DBM, where relative susceptibility in decreasing order was: Final instar larva < prepupa < freshly formed pupa without cocoon < freshly formed pupa with cocoon < two day old pupa without cocoon. More data are needed on the pathogenic and immune response of the nematode within the host body of different developmental stages. One possibility suggested by Finney and Walker (1977) that the resistance of pupa to nematode infection is due to limited routes of entry of the nematode, appears to have a definite role to play in the test insect, DBM. Supporting this view Narayanan and Gopal Krishnan (1987) reported pupae of *S. litura* to be less susceptible than prepupae

and adults to *S. feltiae*. Normally, the nematode IJs enter the insect body through mouth and anus. However, nematode penetration has also been reported through less sclerotized cuticular areas (Kondo and Ishibashi, 1989) and spiracles (Kaya and Hara, 1981; Kondo, 1989). *H. bacteriophora* can infect through the tracheal system (Mracek *et al.*, 1988). Presence of terminal tooth in this nematode species helps it in rapid penetration through cuticle.

Differential susceptibility of various life stages of lepidopterans may be attributed to the differences in the number of portals of entry available for invading nematodes (Kaya, 1985). Pupa is least susceptible stage with only the spiracles as portals for nematode entry. The larvae were more susceptible with mouth and anus as openings beside the spiracles. The present findings are in consonance with above observations.

Susceptibility of DBM adults to nematode infection is an added advantage that can be exploited for better results during DBM management using nematodes. If the nematodes are applied during maximum moth activity, better results may be expected.

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Development and energy flow in the pulse beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) fed on khesari seeds

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ABSTRACT: The development and flow of energy in *Callosobruchus maculatus* (F.) fed on small khesari (*Lathyrus sativus* L. cv Nirmal) seeds were investigated at $27 \pm 6.5\,^{\circ}C$ temperature, $70 \pm 10\%$ relative humidity and 12 ± 1.8 h L photoperiod. Khesari seed contained 12.4% water, 3% ash and 846.1 joules of energy. Egg took 10.3 days for incubation, and 43.5% larvae hatched from incubated eggs. The larval development period decreased with the advancement of instars, as did the instar mortality ($P < 0.05$). The female lived longer ($P < 0.05$) than the male, and deposited 75.6 ± 1.8 eggs in her life-time. The biomass (mg ash-free/insect), and ash (%) and energy (J mg^{-1} ash-free tissue) contents increased linearly with the age of the instar, while water contents showed an inverse relationship. Assimilation efficiency (AE) increased inversely ($P > 0.05$) with the age of the larva, while Gross and Net Production Efficiencies (GPE and NPE) showed linear relationship ($P < 0.05$). Energy contents of the mated and non-ovipositing female and freshly emerged male were highest, and these decreased steadily after egg-laying by the female and mating of the male began. A female used 12.1 and 2.3 J, and a male used 38.8 and 1.8 J for respiration and reproduction, respectively.

KEYWORDS: *Callosobruchus maculatus*, *Lathyrus sativus*, development, fecundity, energy flow

INTRODUCTION

The pulse beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) is one of the several cosmopolitan and polyvoltine insects that infest a number of legume seeds in stores. It breeds regularly within a small khesari (*Lathyrus sativus* L., cv Nirmal) seed which contains high levels of polyphenolics (Bhattacharya, 2000) and potent neurotoxin, β–N–Oxalyl–L–α, β—diaminopropionic acid (ODPA) (Murti *et*

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al., 1964; Rao *et al.*, 1964). But it remains unknown whether prolonged ingestion of these toxic biochemicals in the khesari seed can inhibit the process of development and energy flow among instars, and cause high larval mortality and adverse effects on adult reproduction. The objective of the present investigation is to examine differences in the development and efficiencies of the transfer of energy among instars, and to outline the flow of energy in *C. maculatus* fed on khesari seeds.

MATERIALS AND METHODS

Insect rearing

The khesari seeds used in this study were obtained from local stores for rearing *C. maculatus* at 27 ± 6.5 °C temperature, 70 ± 10% relative humidity and 12 ± 1.8 h L natural photoperiod. Each experimental had three replicates, and each replicate contained 150 khesari seeds and one freshly-mated female in a 250 ml sterilized glass jar. Females were removed from replicate jars three days after initial egg-laying, and all egg-laden seeds were segregated for incubation and hatching. Ten khesari seeds from each replicate with insects developing inside were destructively sampled at regular intervals to determine penetration of the seed-coat by neonate larvae, various life stages, duration of development, mortality, oviposition and energy contents.

Gravimetry and ash contents

The khesari seeds, egg-laden khesari seeds prior to incubation and the left-over seeds at the end of each instar, full grown 1 to 4 instars, pupae, and adults were kept in a hot-air oven at 48 ± 1 °C temperature for 72 h. These oven-dried samples that produced a constant weight, were weighed on an electronic balance (±0.01 mg). Differences in the life and dry weights were used to determine the water contents (%) of the seeds and insects.

A portion (25 ± 5 mg dry tissue/replicate) of all oven-dried samples was placed separately in heat-resistant porcelain crucibles and ashed at 480 ± 20°C temperature for 2 h in a muffle furnace (Sunvic, UK). The ashed samples were weighed in an electronic balance (±0.01 mg), and ash-free weights and ash content (%) of samples were determined by the method outlined by Mahapatra and Banerjee (1990) and Banerjee and Ray (1995). Each of these tests had three replicates and was repeated three times.

Energy transfer and efficiencies

The following equations were used to express ingestion and transfer of the food energy by *C. maculatus* (Petrusewicz and Macfadyen, 1970; Banerjee, 2001).

$$C = P + R + FU \quad (1)$$

$$A = C - FU \quad (2)$$

$$R = A - P \quad (3)$$

where C = consumption, P = production (body growth and exuviae; expressed as energy contents of the insect biomass), R = respiration (metabolism or the cost of maintenance), FU = egestion (F is the part of the food ingested which is egested as faeces, and U represents combined urinary and other excretory products), A = assimilation (the difference between ingestion and egestion). The C, FU and P were measured calorimetrically, and A and R were estimated using equations (2) and (3), respectively.

Efficiencies of energy transfer by 1 to 4 instar *C. maculatus* were estimated using the following ratios:

$$\text{Assimilation efficiency} = (A/C) \times 100 \quad (4)$$

$$\text{Gross production efficiency} = (P/C) \times 100 \quad (5)$$

$$\text{Net production efficiency} = (P/A) \times 100 \quad (6)$$

whenever A, C and P of the individual insects were determined, the instar performance of energy transfer in terms of means and standard errors was estimated from the efficiencies of each insect.

Data analysis

Errors for means are standard errors throughout. One-way analysis of variance (ANOVA) was used to compare differences in the development time, mortality and rates of energy ingestion and egestion of 1 to 4 instars, biomass and energy contents of 1 to 4 instars, pupa and adults. All statistical tests were carried out at $P = 0.05$ level of significance (Bailey, 1965). The efficiency values, measured as percentages, were transformed to arcsine values before the analysis.

RESULTS

Eggs laid on the khesari seed took 10.3 days for incubation and 43.5% larval hatching occurred eventually from the incubated eggs (Table 1). The larval development period and the larval mortality decreased steadily with the advancement of instars ($P < 0.05$). The larval mortality was higher than the pupal or adult mortality. Females, however, lived longer ($P < 0.05$) than males. The female fed on khesari seed deposited 75.6 ± 1.8 eggs during life-time.

Khesari seed had small biomass (64.2 ± 1.2 mg ash-free/seed), water (12.4%), ash (3%) and energy (13.2 J mg^{-1} ash-free tissue) contents (Table 2). The biomass, and ash and energy contents in the tissue increased linearly with the age of instars, whereas water contents showed an inverse relationship with the age of instars.

The rates of energy ingestion and egestion increased linearly until the third instar, and then dropped dramatically at the forth instar (Table 3). In contrast, Assimilation Efficiency (AE) increased inversely with the age of the larva, while the Gross and Net Efficiencies of Production of biomass (GPE and NPE) showed linear relationships with the age of the larva.

TABLE 1. Duration of development, mortality and longevity of *C. maculatus* fed on khesari seeds ($n = 90$; ±1 SE)

Egg incubation		Duration of development							Mortality (%)							No. of
Period	Hatching	Instar					Longevity (day)		Instar					Adult		eggs laid/
(day)	(%)	I	II	III	IV	Pupa	Female	Male	I	II	III	IV	Pupa	Female	Male	female
10.3±	43.5±	12.3±	11.2±	10.4±	8.6±	5.7±	11.2±	5.1±	46.4±	32.3±	16.2±	6.7±	4.9±	2.3±	2.7±	75.6 ± 1.8
0.5	2.4	0.9^1	0.5^1	0.6^1	0.3^2	0.5	1.7^1	0.3^2	3.2^1	2.6^2	1.3^3	0.4^4	0.6	0.2^1	0.2^1	110.2±1.9*
(7–15)		(8–15)	(5–15)	(4–13)	(3–10)	(3–7)	(10–18)	(4–9)								

* Data are from Bhattacharya (2000). Figures enclosed in first brackets correspond to the range. Means having different numerals superscript are significantly different ($P < 0.05$), while comparing one developing stage with the other within a row (ANOVA).

TABLE 2. Differences in the biomass, water, ash and energy contents of the khesari seed and immature stages of *C. maculatus* ($n = 90$; ± 1 SE)

Sample		Biomass (mg dry or mg ash-free/individual)			Water content (%)	Ash content (%)	Energy content (J mg^{-1} ash-free tissue)
		Live weight	Dry weight	Ash-free weight			
Khesari	Seed	75.6 ± 2.1	66.2 ± 1.6	64.2 ± 1.2	12.4 ± 0.6	3 ± 0.2	13.2 ± 0.1
Instar	1	0.08 ± 0.01^a	0.031 ± 0.01^a	0.029 ± 0.01^a	61.2 ± 1.2^a	6.4 ± 0.3^a	19.5 ± 0.5^a
	2	1.7 ± 0.1^b	0.7 ± 0.1^b	0.65 ± 0.1^b	58.8 ± 1.3^a	7.1 ± 0.2^a	24.2 ± 0.8^b
	3	4.3 ± 0.2^c	1.8 ± 0.2^c	1.65 ± 0.2^c	58.1 ± 0.9^a	8.3 ± 0.4^b	29.2 ± 0.5^c
	4	6.4 ± 0.2^d	2.7 ± 0.3^d	2.45 ± 0.3^d	57.8 ± 0.7^a	9.2 ± 0.2^a	34.4 ± 0.6^c
Pupa		6.1 ± 0.3^d	2.6 ± 0.6^d	2.4 ± 0.1^d	57.3 ± 0.9^a	7.6 ± 0.3^a	31.2 ± 0.3^c

Means followed by different letters are significantly different ($P < 0.05$), while comparing one developing stage with the other within a column (ANOVA).

TABLE 3. The energy (J/larva/day) ingestion (C) and egestion (FU), and efficiencies of assimilation (AE), and gross (GPE) and net (NPE) biomass production in 1–4 instar *C. maculatus* fed on khesari seeds ($n = 90$; ±1 SE)

Instar	Ingestion (C)	Egestion (FU)	AE = (A/C) ×100	GPE = (P/C) ×100	NPE = (P/A) ×100
1	4.6 ± 0.1^a	0.6 ± 0.1^a	86.9 ± 2.1^a	9.6 ± 0.2^a	10.7 ± 0.4^a
2	15.1 ± 0.2^b	6.2 ± 0.2^b	84.9 ± 1.8^a	20.1 ± 0.6^b	22.6 ± 0.9^b
3	33.2 ± 0.3^c	9.6 ± 0.2^c	81.5 ± 1.3^a	38.4 ± 0.9^c	47.5 ± 0.7^c
4	25.4 ± 0.2^d	6.3 ± 0.4^b	76.9 ± 2.2^a	66.2 ± 1.2^d	71.6 ± 1.4^d

Means followed by different letters are significantly different ($P < 0.05$), while comparing one instar with other within a column (ANOVA).

The biomass (mg dry or mg ash-free/beetle) and energy content (J mg^{-1} ash-free tissue) of the newly emerged females was greater than those of the newly emerged males (Table 4). The biomass and energy contents of the mature, mated and non-ovipositing female were maximum, and these decreased steadily after egg-laying. In contrast, the biomass and energy contents of the freshly emerged male were maximum, and these decreased steadily after mating.

DISCUSSION

The development of various stages of *C. maculatus* was considerably influenced by small khesari seeds (Table 1). Larvae ingested concentrated food materials and water in seeds. And the pupa and adults do not eat, but solely depend on organic materials and water stored to complete the life cycle (Wightman, 1978b; Tohiuddin *et al.*, 1993). The toxic compound, ODPA present in khesari was found to affect the growth of the insect larva, *Corcyra cephalonica* Staint. by inhibiting transmission of aspartate (Rao *et al.*, 1964). The ingestion of khesari seeds by *C. maculatus* larvae caused higher mortality (16.2–46.4%) among early three instars. Further, the duration of development of this bruchid fed on khesari was longer (63.6–69.7 days) implicating that the species may invite risks of natural enemies (e.g. parasitoids, pathogens, etc.) and complete fewer generations.

Unlike other holometabolan insects, a *C. maculatus* larva fed continuously on a single khesari seed to complete development and maximize the concentration of energy in its tissue for reproduction during the adult stage (Table 2). Tohiuddin *et al.* (1993) demonstrated that the rates of ingestion of food energy from chickpea (*Cicer arietinum* L.) by a closely related species, *C. chinensis* increased linearly with the age of the larva, as did the rates of excretion and assimilation of energy in the biomass. In contrast, the rates of energy ingestion and egestion in this study showed different trends, and the energy ingestion (C) and egestion (FU) were maximum in the third instar (Table 3). On the other hand, the assimilation of energy (J mg^{-1} ash-free tissue) and cumulative production of biomass were maximum in the fourth instar. It is likely

TABLE 4. The biomass and energy contents in *C. maculatus* adults fed on khesari seeds ($n = 90$; ± 1 SE)

Age and status of beetle	Biomass (mg dry or mg ash-free/beetle)				Energy content (J mg^{-1} ash-free tissue)	
	Dry weight		Ash-free weight			
	Female	Male	Female	Male	Female	Male
Newly emerged (0–2h; non-mated beetles)	2.4 ± 0.2^{a1}	1.5 ± 0.2^{a2}	2.3 ± 0.1^{a1}	1.4 ± 0.1^{a2}	27.3 ± 0.4^{ab1}	25.8 ± 0.5^{a1}
Mature and mated (2–6 h; mated male and non-ovipositing mated female)	2.6 ± 0.2^{a1}	1.3 ± 0.2^{ab2}	2.5 ± 0.1^{a1}	1.2 ± 0.2^{a2}	31.1 ± 0.3^{a1}	20.5 ± 0.4^{b2}
Mature and mated (6–192 h; ovipositing mated female)	2.2 ± 0.1^{ab}	–	2.1 ± 0.1^{ab}	–	23.4 ± 0.4^{bc}	—
Senile and non-mating (288–360 h; male and non-ovipositing female)	1.9 ± 0.1^{b1}	1.1 ± 0.1^{b2}	1.8 ± 0.1^{b1}	0.9 ± 0.1^{b2}	21.2 ± 0.2^{c1}	16.8 ± 0.6^{c2}

Means followed by different letters or having different numerals superscript are significantly different ($P < 0.05$), while comparing one status or one sex of the beetle with the other within a column or within a row, respectively (ANOVA).

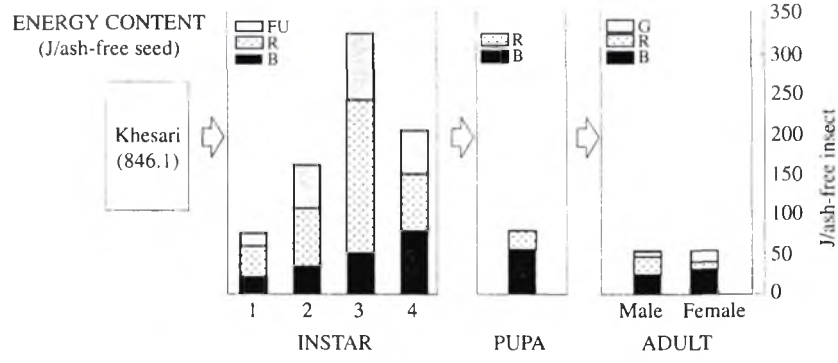

FIGURE 1. A schematic model for the flow of energy in *C. maculatus* fed on Khesari seeds. Rectangles enclosing histograms indicate the transfer of energy at respective levels (FU = egestion, R = respiration, B = biomass and G = reproduction).

that the fourth instar, compared to other 3 instars, entered into the state of sluggishness which may cause fewer ingestions and greater storage of organic materials.

Energy in this species is considered as flowing through four gross compartments, namely, khesari seeds, instars, pupa and adult (Fig. 1). An ash-free khesari seed contained 846.1 J, and 93% of which were ingested by the four instars during development. Most of the ingested energy (i.e. 81.1%) were lost through faeces, exuviae and respiration, and 18.9% were actually converted into larval biomass. For maintenance and metamorphosis into adults, a *C. maculatus* pupa expended 12.9% of the energy accumulated at the fourth instar. These results partly agree with those of Wightman (1978a) and Tohiuddin *et al.* (1993), who demonstrated that the extent of energy used for metabolism and metamorphosis by *C. analis* and *C. chinensis* pupae fed on dwarf green pea (*Pisum sativum* L.) and chickpea (*C. arietinum* L.) respectively, ranged from 25 to 35 J. Furthermore, a newly emerged *C. maculatus* male or female had 74.9 J, of which 51.8 and 16.2% were used for respiration, and 48.2 and 83.8% were retained in the biomass for reproduction and storage in cadavers.

A survey of the relevant literature (e.g. Wightman, 1978b; Tohiuddin *et al.*, 1993) on the energetics of *C. analis* and *C. chinensis* fed on dwarf green peas and chickpeas respectively indicated that energy contents in these beetles were high. Nevertheless, the size, energy contents and levels of toxic biochemical in khesari seeds, compared to dwarf greenpeas and chickpeas, may be the probable reasons for fewer energy contents in *C. maculatus* beetles, and as such has a reflection on the number of eggs produced (Table 1).

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Two little known genera of Encyrtidae (Hymenoptera : Chalcidoidea) from India, with description of two new species from Assam

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ABSTRACT: The encyrtid genus *Epiblatticida* Girault is recorded for the first time from India, a new species, *E. psyllidiphaga*, is described from specimens bred from psyllid, *Mesohomotoma hibisci*. A new species *Bothriothorax kazirangaensis* is also described.

KEYWORDS: Hymenoptera, Encyrtidae, *Epiblatticida psyllidiphaga* sp. nov., *Bothriothorax kazirangaensis* sp. nov.

INTRODUCTION

This paper deals with a small collection of encyrtids made mainly in Assam. This material belongs to the genera, *Epiblatticida* Girault and *Bothriothorax* Ratzeburg.

The genus *Epiblatticida* is represented so far by 5 species, all from Australian region: *aereitibiae* (Girault), *argentipes* (Girault), *caudatus* (Girault), *lambi* Girault, and *minutissimus* (Girault) (see Noyes and Hayat, 1984; Noyes, 1988; Dahms and Gordh, 1997). This genus is recorded here for the first time from India, and a new species is described.

The genus *Bothriothorax* contains 30 species described mainly from the Holarctic region. The presence of this genus in India was reported by Noyes and Hayat (1984). A new species of this genus is described here from Assam.

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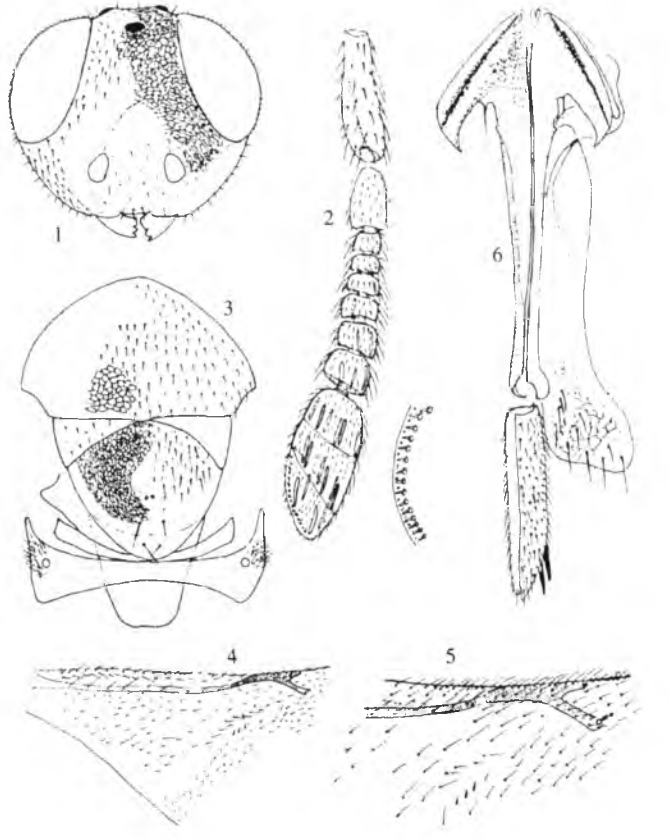
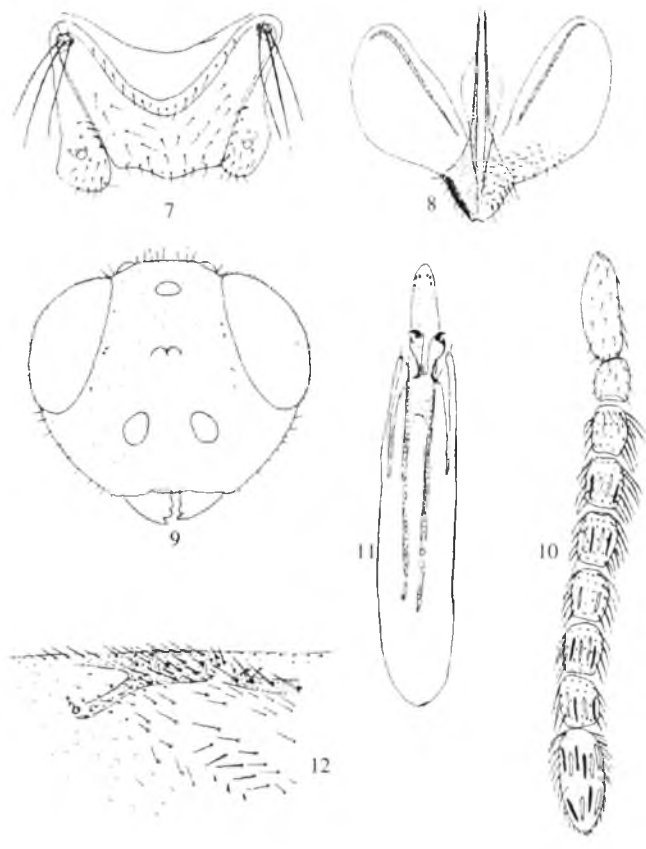


FIGURES 1–6. *Epiblatticida psyllidiphaga* sp. nov. female: 1, head in frontal view; 2, antenna; 3, thorax; 4, fore wing showing setation; 5, fore wing venation; 6, ovipositor.

***Epiblatticida psyllidiphaga* sp. nov. (Figs 1–12)**

Female

(Figs 1–8): Length (excluding exserted part of ovipositor), 1.22 ± 0.13 mm, ($n = 7$). Holotype: 1.38 mm long. Body completely brown, faintly metallic; head dull green dorsally (frontovertex); below frontovertex along the eye, malar space and antennal prominence with blue and purple reflections; frontovertex with small punctures, half to three-fourths of the middle ocellus diameter apart, with small pale setae; below frontovertex the punctures are not as concentrated, with slightly more white setae; scrobes deep, meeting dorsally with rounded margins. Antenna straw colored; scape, pedicel and clava in some specimens (including holotype) with brownish tinge. Sculpture on the frontovertex very shallow, reticulate. Eyes dark brown, with small setae (visible at higher magnification). Pronotum, mesoscutum and tegulae dull green with bronzy reflections and shallow reticulately sculptured; scutellum more greenish



FIGURES 7–12. (7–8)—*Epiblatticida psyllidiphaga* sp. nov., female: 7, last tergum of gaster; 8, hypopygium. (9–12)—Male: 9, head in frontal view; 10, antenna; 11, male genitalia; 12, venation of fore wing.

with reddish brown reflections, deeply reticulate on the most part except the posterior portion and side stripes which are glabrous and more bronzy; mesopleuron, on about anterior two-thirds, green and with reticulate sculpture, rest violet bronzy; lateral sides of propodeum green and with 25–30 pale setae. Gaster with basal tergite metallic deep green and glabrous; ovipositor sheath brown. Wings hyaline, venation brownish. Legs light yellow except light to moderately brown mid- and hind-coxae; pre tarsi darker.

Head (Fig. 1) with frontovertex narrowest at posterior ocelli, one-third of head width; head width 2.8× the frontovertex width at anterior ocellus level (28 : 10); ocellar triangle with apical angle slightly acute, posterior ocelli about 0.5× the diameter of an ocellus from eye margin and slightly more than 0.5× diameter to occipital margin; eyes over-reaching occiput behind; occiput concave from side to side and with upper margin sharp. Mandible with two small teeth and a truncation.

Antenna as in Fig. 2 (note the oblique second suture of clava with a ventral sensory area).

Thorax (Fig. 3) with scutellum, strongly convex; mesoscutum about 1.6× as broad as long and slightly longer than scutellum; scutellum slightly broader than long; mesopleuron not enlarged posteriorly, so that the hind coxa is in contact with metapleuron. Fore wing venation and discal setation as in Figs 4 and 5. Mid tibial spur slightly shorter than basitarsus.

Gaster slightly shorter than thorax; T-VII with apex broad (Fig. 7); ovipositor (Fig. 6) exserted part slightly less than 0.33× of gaster: hypopygium (Fig. 8) extending past apex of gaster.

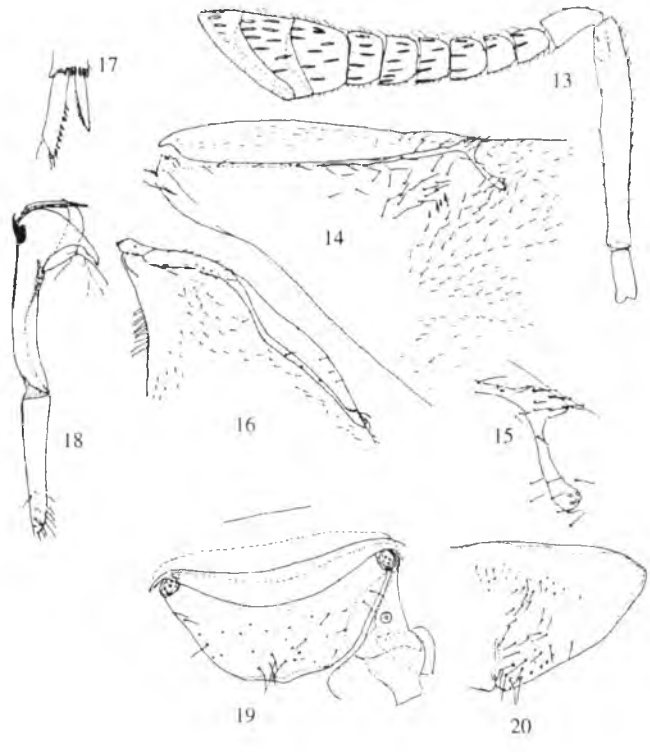
Relative measurements (From carded Holotype): Head dorsal width, 28; head frontal length: width in frontal view (25 : 28); frontovertex width at anterior ocellus level, 10; intertorular distance, 4 ; Eye length 16, width 13; distance between posterior ocelli, 3.5; distance from a posterior ocellus to eye margin, 1.5; distance form a posterior ocellus to occipital margin, 1.5; distance form a posterior ocellus to anterior ocellus, 4. Thorax length, 37; mesoscutum length, 17; width, 28; scutellum length, 15; width 16. Gaster length, 28; hypopygium extended beyond tip of gaster, 5; exserted part of ovipositor, 10. From slide (paratype): torulo-eye-distance 16; torulo-mouth-distance 8; inter torular distance 17; mouth fossa width, 29; scape length: width, 26: 7.5. Fore wing length, 180; width 80; marginal vein 10, post-marginal 5; stigmal 8, costal cell length 70, width 6; Lengths: mid tibia, 78; mid basitarsus, 22; mid spur, 18; ovipositor length 112, third valvula length 37.

Male

(Figs 9–12): Similar to female in coloration except that the facial area below the frontovertex metallic green; middle and hind coxae dark brown; gaster with basal tergite bronzy.

Head (Fig. 9) with frontovertex 2.3× as wide as at anterior ocellus level; posterior ocelli about 0.3× the diameter of an ocellus from eye margin, about 0.5× the diameter of an ocellus from occipital margin; toruli on the line joining lower margins of the eyes. Antenna as in Fig. 10. Mesoscutum 1.86× as broad as long (28 : 15); scutellum slightly shorter than mesoscutum, as long as wide (16). Fore wing venation and discal setae as in Fig. 12. Male genitalia as in Fig. 11.

Relative measurements: Head dorsal width, 28; frontovertex width at anterior ocellus level, 12; eye length 15.5, width 12; distance between posterior ocelli 5; distance from a posterior ocellus to eye margin 0.5; distance from a posterior ocellus to occipital margin 1; distance from post ocellus to anterior ocellus 5. Thorax length 35, mesoscutum length 15, width 28; scutellum length 16, width 16. Gaster length 25. From slide: torulo-eye-distance 16; torulo-mouth-distance 15; mouth fossa width, 29; scape length, 24. Fore wing length, 190; width 90; marginal vein 12, post-marginal 10; stigmal 11, costal cell length 77, width 7; hind wing length 140, width 41; vein length 93; mid tibia, 82; mid basitarsus, 23; mid spur, 19. Aedeagus length, 65.



FIGURES 13–20. *Bothriothorax kazirangaensis* sp. nov., female, holotype: 13, antenna; 14, fore wing showing proximal setation and with distal veins enlarged; 15, fore wing showing venation; 16, part of hind wing; 17, mid basitarsus and spur; 18, part of ovipositor, left half; 19, last tergum of gaster; 20, hypopygium, left half.

Host: Hyperparasitoid of the psyllid, *Mesohomotoma hibisci* (Froggatt) on leaves of *Bombax cieba* through encyrtid *Psyllaephagus mesohomotoma* Singh & Agarwal.

Distribution: India: Assam, Uttar Pradesh.

Holotype: ♀ (on card); India: Assam: Silchar, 08.xii. 1994, hyperparasitoid of psyllid, *Mesohomotoma hibisci* (Froggatt) on leaves of *Bombax cieba* through encyrtid *Psyllaephagus mesohomotoma* Singh & Agarwal, coll. Sudhir Singh.

Paratypes: 10 ♀, 8 ♂ (2 ♀ and 1 ♂ on slides); data same as for holotype. 8 ♀, 6 ♂ (1 ♀, 1 ♂, on slides), 20.ix.1998, other data as for holotype; 1 ♀ (on slide under 4 coverslips), Uttar Pradesh, Aligarh, 25. ix. 1988, with *Mesohomotoma hibisci* on leaves of *Bombax cieba*, coll. Sudhir Singh (coll. No. 115); Aligarh specimen and 2 ♀, 1 ♂ in Hayat collection (Aligarh); holotype and remaining paratypes deposited in

National Insect Reference Collection, Forest Entomology Division, Forest Research Institute, Dehradun. Accession No. 20971.

Comments

This new species is very close to *E. lambi* Girault in leg colour, but differs in several characters: width of frontovertex about one-third of head width; antenna with funicle segments gradually increasing in length and width distad; pedicel as long as F1-3 combined; exserted part of ovipositor nearly 1.5× as long as mid tibia; and third valvula about 2× as long as mid tibial spur. (In *lambi*: frontovertex 0.38× of head width; antenna with F1 and F2 clearly shorter than F3; pedicel shorter than F1-3 combined; exserted part of ovipositor 0.47× of gaster length; ovipositor 1.8× as long as mid tibiae; and third valvula 2.79× as long as mid tibial spur. See Dahms and Gordh, 1997).

***Bothriothorax kazirangaensis* sp. nov. (Figs 13–20)**

Female

Length, 2.05 mm. Body dark brown to black, metallic shining; head with large, deep, intense bluish green, thimble-like setigerous punctations up to malar sulci; about three rows of punctures extending between toruli and malar sulcus, and these punctures reddish bronzy violet; the raised ridges between punctures bronzy; head behind malar sulcus with fine, longitudinal reticulations with bluish green shine; inter-torular space with small punctures; mouth margin violet bronzy; pronotum, including collar, bluish green with some bronzy lustre, sides violet bronzy; tegulae dark brown; mesoscutum with punctures deep and similar in size to those on frontovertex, intense bluish green, anteriorly with bronzy violet lustre; axillae bluish green to violet, each with 5–6 small punctures; scutellum in about basal two-thirds as on mesoscutum, distal one-third with raised lines (ridges) and shallow punctures, and with intense violet bronzy shine; declivous margins of scutellum nearly smooth: propodeum with a median ridge and submedian ridges (plicae) on each side, the area between the submedian ridges smooth and shining bluish green to violet bronzy; nucha distinct, transverse, and with short longitudinal ridges; distad of submedian ridges smooth and densely setose, the setae white; prepectus bluish green, with raised reticulate sculpture, and white along margins; mesopleuron violet; gaster dark brown, nearly completely with violet shine, mixed with some reddish bronzy. Antennal radicle and scape testaceous yellow, scape with dorsal margin pale infuscate; pedicel and flagellum reddish brown. Wings hyaline. Legs: all coxae and mid trochanter dark brown, metallic; fore and hind trochanters, all femora, fore and mid tibiae, tarsal segments 1–4 of fore legs, testaceous yellow; fifth segments of fore and hind tarsi, last 2–3 of mid tarsus, dark brown; hind tibiae in distal two-thirds and tarsal segments 1–4, infuscate brown to reddish brown. Setae on head and thoracic dorsum short and brown.

Head nearly menisciform; occiput concave from side to side and with upper margin sharp; ocelli forming a flat triangle, posterior ocellus separated from occipital margin; scrobes shallow, each shorter than torulus length. Antenna as in Fig. 13. Mesothoracic

dorsum strongly convex. Fore wing with setation and venation as in Figs 14 and 15; setae distad of linea calva short and hyaline. Hind wing with costal cell well-developed (Fig. 16). Mid tibial spur distinctly shorter than basitarsus (Fig. 17). Gaster shorter than thorax and as long as broad; hypopygium reaching nearly to apex of gaster. (Gaster removed, dissected and mounted on slide.) Ovipositor, last tergum and hypopygium as in Figs 18–20.

Relative measurements: (from carded holotype): head dorsal width, 65; frontovertex width, 33.5 (scape length, 34); head frontal height, 58; torulus length, 10; torulus mouth-margin distance 10; distance between toruli, 9; eye length, 32; malar space length, 24; distance between posterior ocelli, 21; distance from a posterior ocellus to eye margin, 3; distance from a posterior ocellus to occipital margin, 2; distance from a posterior ocellus to anterior ocellus, 10.5. Thorax length, 92; pronotum (visible part) length, 6; mesoscutum length (width), 46 (68); scutellum length (width), 44 (38); propodeum median length, 10; distance between propodeal spiracles, 55. Mid tibia length, 60; mid basitarsus length, 19; mid spur length, 13.5. Gaster length (width), 60 (60); T-I length, 28; cercal plates from base of gaster, 46. (From slide): Fore wing length (width), 275 (120); hind wing length (width), 175 (71). Ovipositor length, 63; third valvula length, 27; T-VII length, 33; mid tibia length, 100; mid basitarsus length, 32; mid spur length, 22.5.

Male: Unknown.

Host: Unknown.

Distribution: India: Assam.

Holotype: ♀ (on card, with left antenna, wings, legs and dissected gaster on a slide): India: Assam, Kohra near Kaziranga National Park, 24. Iv. 1988, coll. Sudhir Singh, (coll. No. 105). Deposited in National Insect Reference Collection, Forest Entomology Division, Forest Research Institute, Dehradun; Accession No. 20972.

Comments

The new species, *B. kazirangaensis*, comes close to *B. icelos* Trjapitzin (1967) and agrees with it in a majority of characters pertaining to sculpture and colour; see also Trjapitzin (1989), but differs as follows: antennal scape nearly 8× as long as broad with a thin apical expansion; truncate part of clava at least 2× of rest of ventral margin: scutellum not longer than mesoscutum; propodeum with hind corners without a tooth; gaster two-thirds length of thorax; fore wing with about 20 setae proximad of linea calva. (In *icelos*: scape 4× as long as broad, with a distinct expansion in distal third; truncate part of clava 1.5× of rest of ventral margin; scutellum slightly longer than mesoscutum (7 : 6); propodeum on each hind corner with a tooth; gaster slightly shorter than thorax (13 : 15); fore wing with 9 setae proximad of linea clava.)

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Studies on Anopheline mosquitoes of tribal and non-tribal areas in Bankura district, West Bengal, India

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ABSTRACT: Present study was conducted in some tribal and non-tribal areas of Bankura district, West Bengal, India from August 1997 to July 1999. Both indoor-resting and man-biting *Anopheles* mosquitoes were collected. Three species namely *Anopheles subpictus*, *A. vagus* and *A. barbirostris* were available in tribal areas. In non tribal areas, besides above three species. *A. annularis* were collected. Seasonal variations and man hour density of indoor-resting population were recorded. In case of man-biting population, peak biting hour, man-hour density, seasonal variations in biting activity and per man per night density were recorded. No infected (with *Plasmodium*) *Anopheles* was obtained in the study area.

KEYWORDS: Anopheline mosquitoes, tribal and non-tribal areas, West Bengal.

West Bengal is endemic for malaria. Malarial endemicity in West Bengal is very high, specially in Calcutta, Ayodhya hills and foot hills, districts of North Bengal etc. Recently *P. falciparum* malaria assumed its gigantic nature in different parts of the state. Hospital data of Bankura district revealed a high range of malaria cases throughout the year but no work on its related vector has been conducted so far in this area. Present study has been designed to observe the species composition, seasonal prevalences and man-hour density of indoor-resting and man-landing Anophelines and peak biting hour and per man per night density of man-biting population of *Anopheles* species available in some tribal and non-tribal areas of Bankura district of West Bengal. Anopheline population was examined (salivary glands and guts) to incriminate the responsible malaria vector in the study areas.

The tribal areas were situated within the dense or moderate forests, totally isolated from non-tribal study areas namely Sonamukhi Municipal town of Bankura District.

Indoor-resting Anophelines were captured from 80 human habitations of 8 tribal villages (10 habitations from each village) and from 80 human habitations of 8 localities (10 habitations from each locality) of non-tribal areas. Anophelines were

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collected for 12 minutes from each habitation i.e. for 120 minutes from 10 habitations in alternate weeks from a tribal village and a non-tribal locality following the methodology suggested by WHO (1975) and De and Chandra (1994). The study was conducted from August 1997 to July 1999 i.e. for two consecutive years.

Anophiline mosquitoes were collected off man-baits placed at indoors and outdoors throughout the night from 6 p.m. to 6 a.m. fortnightly for one year from August 1997 to July 1998 in both tribal and non-tribal study areas in an alternate manner. The experiment was conducted following the method of Service (1976) employing 288 man-hours in each location of indoor and outdoor in each area.

Salivary glands and guts of all indoor-resting and man-landing, Anophelines were, dissected and examined for the detection of malarial parasites.

In tribal area, Anophelines comprised of 33.15% (742) of the total indoor-resting mosquitoes of different species (2238) and it was 8.65% (602) in non-tribal area out of total 6955 mosquitoes of different species. In tribal areas average prevalence of Anophelines were 45.4, 35.9 and 18.7% in rainy season, winter and summer respectively. Only three species of Anophelines were detected namely *A. subpictus* (138; 6.16%), *A. vagus* (432; 19.3%) and *A. barbirostris* (172; 7.68%). The man-hour densities were 1.44, 4.50 and 1.80 respectively. In non-tribal areas average densities of Anophelines were 65.4, 20.6 and 14.0% in rainy season, winter and summer respectively. Altogether 4 species of indoor resting Anophelines were detected namely *A. subpictus* (110; 1.58%), *A. annularis* (10; 0.14%) *A. vagus* (390; 5.60%) and *A. barbirostris* (92; 1.32%) and their man-hour densities were 1.14, 0.10, 4.06 and 0.96 respectively.

In case of man-landing catches, out of altogether 5959 mosquitoes, 29.23% (1742) were Anophelines in tribal area and out of 19018 mosquitoes in non-tribal areas the Anophelines comprised of 8.19% (1557). In the tribal area 3 species namely *A. subpictus* (202; 3.4%) *A. vagus* (1379; 23.14%) and *A. barbirostris* (161; 2.7%) were available and their man-hour densities were 0.35, 2.4 and 0.28 respectively. In non-tribal zone, the available species were *A. subpictus* (159; 0.84%), *A. vagus* (1265; 6.65%). *A. barbirostris* (125; 0.66%) and *A. annularis* (8; 0.04%) and their man hour densities were 0.27, 2.2, 0.21 and 0.01 respectively (Table 1).

In non-tribal belt, peak biting hours of *A. subpictus* and *A. annularis* was between 1 a.m. and 2 a.m. and it was between 11 p.m. and 12 mid night for *A. vagus* and between 9 p.m. and 10 p.m. for *A. barbirostris*. In tribal belt, the peak biting hours was between 10 p.m. and 11 p.m. for *A. subpictus* and *A. vagus* and it was between 9 p.m. and 10 p.m. for *A. barbirostris*. Per man per night density of each species of both the areas has been presented in Table 1. Out of total Anophelines collected on man-baits in tribal areas 48.0, 33.7 and 18.3% came to bite in rainy, winter and summer seasons respectively and the figures for the non-tribal study areas were 61.6, 23.8 and 14.6% respectively. No *Anophelines* mosquito was found to be positive for malarial parasites.

All the anophelines species were more prevalent in the rainy season both in tribal and non-tribal areas and majority of them came to bite human in this season. Peak biting hour of Anophelines was earlier in the tribal area than that of non-tribal area. Among both indoor-resting and man-biting anophelines most prevalent species was

TABLE 1. Per man-hour density (PMD) of indoor-resting and man-biting Anophelines, their peak biting hour (PBH) and density per man per night (PMN) in tribal and non-tribal areas of Bankura district, West Bengal

Name of Anophelines	Tribal area				Non tribal area			
	PMD (Indoor resting)	PMD (Man-biting)	PBH	PMN	PMD (Indoor resting)	PMD (Man-biting	PBH	PMN
A. subpictus	1.44	0.17	10–11 p.m.	4.21	1.14	0.14	1–2 a.m.	3.31
A. vagus	4.50	1.20	10–11 p.m.	28.73	4.06	1.10	11–12 p.m.	26.35
A. barbirostris	1.80	0.14	9–10 p.m.	3.35	0.96	0.11	9–10 p.m.	2.60
A. annularis	0	0	-	0	0.10	0.007	1–2 a.m.	0.17

A. vagus in both tribal and non-tribal study areas. The percentage of Anophelines with regard to other genera was significantly higher ($P < 0.05$) in tribal area than that of non-tribal area. It is well known that Anophelines prefer to breed in fresh water ponds, rice-fields, tanks, roof gutter, artificial containers and any temporary collection of water (Covell, 1944; Bhatt *et al.*, 1991; Rajnikant *et al.*, 1993). The presence of higher number of fresh water breeding sources (i.e. ponds, rice fields etc.) in the tribal areas was the probable cause of higher density of Anopheline fauna in this area. Though malaria vector could not be detected in the present study area, present study may help to formulate a mosquito control strategy. *A. subpictus* has been incriminated as a vector of malaria in some parts of the world like Australian zone (Russel *et al.*, 1963); Celebes (Van Hell, 1952), Portuguese timor (Ferreira and Breda, 1962), Coastal areas of South India (Panicker *et al.*, 1981), West Bengal (Chatterjee and Chandra, 2000), Madhya Pradesh (Kulkarni, 1983). *A. annularis* has also been reported as a carrier of malaria in Hooghly district of West Bengal (Ghose *et al.*, 1985).

The density of vector is one of the major factors affecting the epidemology of malaria. It determines the degree of contact between man and the vector and thereby intensity of malaria transmission. The higher prevalence of anopheline fauna and high range of man-hour density in tribal areas are noteworthy from the epidemiological standpoint. Further studies are necessary to incriminate the malaria vector therein.

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Cell mediated immune reactions in 5th larval stage of *Ropalidia marginata* (Hymenoptera: Vaspidae)

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ABSTRACT: The present study deals with various types of haemocytes found in the 5th larval stage of *Ropalidia marginata* and their role in cell mediated immune reaction that is phagocytosis, nodule formation and encapsulation.

KEYWORDS: *Ropalidia marginata*, cell mediated immunity, haemocytes.

The immune system in insects is different from vertebrates as they lack an antigen-antibody complex, although they are capable of responding very effectively against the various foreign invaders. A number of workers have described the role of haemocytes in the defence mechanism of arthropods (Ratcliffe , 1982; Gotz, 1986; Pathak, 1993). 5th instar Larvae of *Ropalidia marginata* were collected from their nests placed at varandas of houses and from other shelter places.

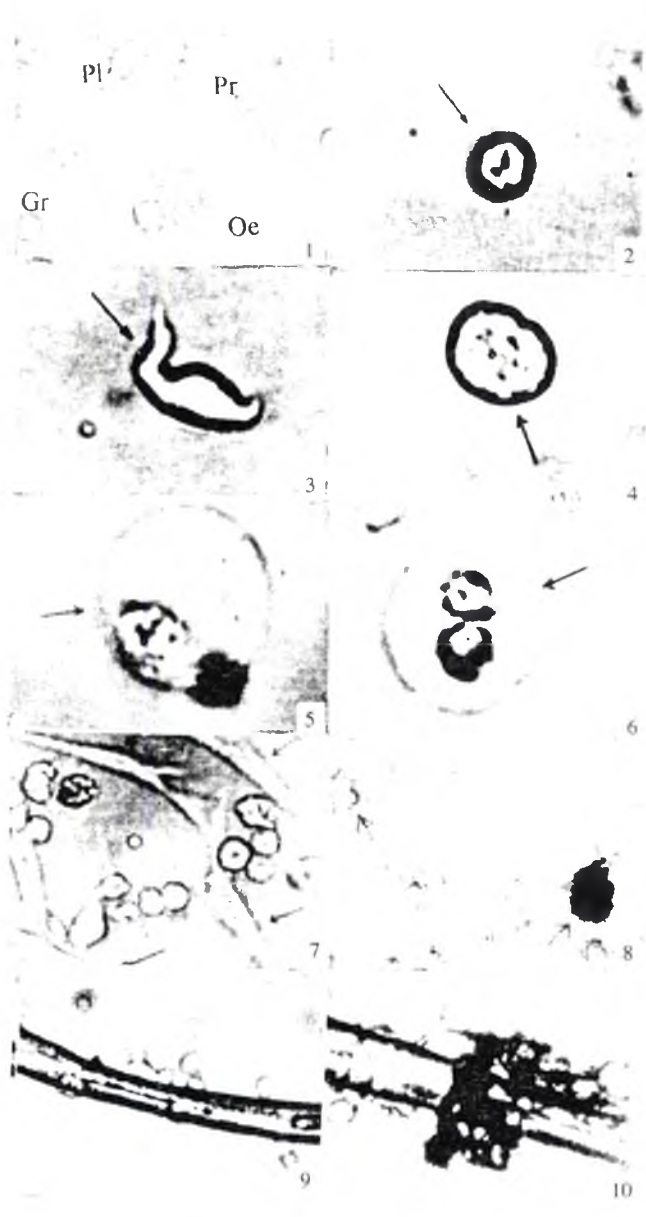
To study the structure of haemocytes, smears were stained by Giemsa and Wright's stain, the hanging drops of haemolymph were also observed in phase contrast microscope.

To study the phagocytosis 1 μl solution of the spores of *Aspergillus niger* (1×10^5 spores/ml in Ringer's solution) was injected in the haemocoel of 5th instar larvae, while for the study of nodule formation 2 μl spore solution was injected. The fluid (injected solution and haemolymph) was withdrawn from the insects at regular intervals to study the reaction of haemocytes to the foreign bodies. To study the encapsulation minute sterilized glass rod and silk filaments were used.

In the present study four types of haemocytes were identified in 5th instar larvae of *Ropalidia marginata*. They were prohaemocytes, plasmatocytes, granular cells and oenocytoids (Fig. 1).

Prohaemocytes are stem cells, they are small rounded or oval in shape (Fig. 2). It is considered that they modify into plasmatocytes and other types of haemocytes (Jones, 1962). They also show mitotic division (Gupta and Sutherland, 1966; Gupta, 1979; Brehelin and Zachary, 1986). However, in the present study no mitotic figures were observed.

Plasmatocytes are ovoid or spindle shaped structure they usually show one or two pseudopodia or protoplasmic extensions, their cytoplasm is basophilic (Fig. 3). The



FIGURES 1–10: 1. Haemocytes of 5th instar larva of *Ropakidia marginate* (400X). Pr = Prohaemocyte, Pl = Plasmatocytes, Gr = Granular cell, Oe = Oenocytoid. 2. Prohaemocyte (600X). 3. Plasmatocyte showing pseudopodia (400X). 4. Large granular cell (600X). 5. Enlarged single nucleated Oenocytoid (600X). 6. Enlarged binucleated Oenocytoid (600X). 7. Spreaded plasmatocytes showing phagocytosis of Spores (600). 8. Nodule formation of *Aspergillus niger* spores (400X). 9. Early stage of encapsulation, arrow indicated the adherence of granular cells (100X). 10. Melanization of encapsulated glass rod (400X).

most important character of the plasmatocytes is its spreading behavior on glass surface.

The third important haemocytes are granular cells. They posses fine acidophilic granules in their cytoplasm. These cells were classified into four sub types on the basis of electron microscopic study (Brehelin and Zachary, 1986). However in the present study using light microscopy only two types of granular cells were distinguished on the basis of size (Fig. 4).

Oenocytoids, were large with low nucleocytoplasmic ratio. They were very stable cells and may be single nucleated (Fig. 5) or binucliated (Fig. 6). They probably produce prophenoloxidase enzyme (Essawy *et al.*, 1985 and Ashida *et al.*, 1988).

In the present study cell mediated immune reactions were studied *in vivo* and *in vitro* in 5th instar feeding larvae of *Ropalidia marginata*. The plasmatocytes are the main phagocytic haemocytes (Fig. 7). A number of workers have reported the active involvement of plasmatocytes and granular cells in phagocytosis (Ratcliffe and Rowley, 1975; Wago, 1983; Pathak, 1993). However, in the present study, phagocytosis was observed only in plasmatocytes.

The degenerating granular cells are involved in the process of nodule formation. The spores are entrapped in the coagulum produced by granular cells and the entire complex changed into a large nodule (Fig. 8), which is isolated from the circulation. These nodules further get encapsulated by plasmatocytes (Vey, 1968, 1969; Vey *et al.*, 1968; Ratcliff and Gagen, 1976; Pathak, 1993).

The large foreign bodies such as glass rod or allografts (silk filament) are encapsulated by coagulum of granular cells and surrounded by plasmatocytes as shown in fig. 9. The granular cells reach to glass rod and release the sticky substance, which attracts the plasmatocytes to form a capsule. The capsule is melanized within 22–24 hours after encapsulation (Fig. 10).

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Determination of dosage levels of *Steinernema bicornutum* and *Heterorhabditis indica* (Rhabditida) for *in vitro* use against *Agrotis ipsilon* Hüfnagel (Noctuidae: Lepidoptera)

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ABSTRACT: The median lethal concentrations of *Steinernema bicornutum* (PDBC EN 3.2) and *Heterorhabditis indica* (PDBC EN 13.3) against *Agrotis ipsilon* were worked out to be 64 infective juveniles (IJs) ($Y = 8.911X + 0.2557, r^2 = 0.7364$) and 34 IJs per larva ($Y = 8.2843X + 1.3603, r^2 = 0.7581$), respectively at 72 h post inoculation by dose response assay. Time assay response with the same revealed LT_{50} values of 72 ($Y = 0.1033X - 2.4466, r^2 = 0.9113$) and 58 h ($Y = 0.1064X - 1.1283, r^2 = 0.7442$), respectively. A positive correlation was obtained with the nematode concentrations and time for mortality. *H. indica* required less number of IJs and lesser time for bringing desired mortality of *A. ipsilon*. The role of dose determination in biological control programmes is discussed.

KEYWORDS: *Steinernema bicornutum*, *Heterorhabditis indica*, *Agrotis ipsilon*, LC_{50}, LT_{50}.

The entomopathogenic nematodes of the family Steinernematidae and Heterorhabditidae are pathogenic to a wide range of insect pests including cutworms (Poinar, 1986; Singh, 1982). Various laboratory and field experiments have been undertaken with different strains of EPN against *Agrotis* spp. (Lossbroek and Theunissen, 1985; Capinera *et al.*, 1988). Dosage levels in the range of 1–200 IJs per larva have been used so far in all the experiments and the true picture of relative efficacy of nematode strain against particular species of *Agrotis* is not available. Estimation of LC_{50} is a relative measure of susceptibility of a host population and is convenient and commonly used index of relative efficacy (Epsky and Capinera, 1994). A positive correlation between nematode concentration and host mortality enables estimation of LC_{50}. Hence an attempt is made to study the correlation between nematode concentration, host mortality

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TABLE 1. Multiple correlation between the concentrations of *S. bicornutum* and *H. indica* and time for mortality

Time for mortality	Concentration	
	S. bicornutum (PDBC EN 3.2)	*H. indica* (PDBC EN 13.3)
Time 1 (24 hrs)	—	0.158111
Time 2 (72 hrs)	0.867926[a]	0.858121[a]
Time 3 (96 hrs)	0.812999[a]	0.870712[a]

[a] Table values—0.3976 (5% level of significance) and 0.5069 (1% level of significance).
Values are highly significant at 5% and 1% level of significance.

and time for mortality and to determine LC_{50} for *Steinernema bicornutum* PDBC EN 3.2 and *Heterorhabiditis indica* PDBC EN 13.3 by dose response and time exposure assays.

The indigenous entomopathogenic nematode *S. bicornutum* (PDBC EN 3.2) and *H. indica* (PDBC EN 13.3) were reared on final instar larvae of the greater wax moth, *Galleria mellonella* based on the methods of Woodring and Kaya (1988). The nematodes were stored in distilled water at 8 °C (*S. bicornutum*) and 24 °C (*H. indica*) and then acclimatized at ambient temperature (25–28 °C) for 24 h before use. *A. ipsilon* larvae were used for experiments. The dose-response assay included namatode concentrations of 0, 25, 50, 75 and 100 IJs/larva; the assay was conducted in sand column using plastic containers (50 × 80 mm) filled with sterile sand (10% moisture w/w) to a depth of 4 cm. The larval mortality was recorded 24, 72 and 120 h post inoculation (time-response assay). Each treatment ($n = 12$) was replicated five times.

The mortality values were converted to Probit mortality and nematode concentrations were transformed to log values. The LC_{50} and LT_{50} values were calculated using regression analysis. Correlation between nematode concentration and time for mortality were worked out and all comparisons were made at the 0.05% level of significance.

At 24 h after inoculation there was no mortality of *A. ipsilon* even at the highest dose level of 100 IJs/larva (Fig. 1). Significant differences in mortality were observed in both the nematode isolates used >50 IJs/larva. The mortality increased with increase in time of exposure. Singh (1993) reported the increase in mortality of insect larvae with time. The mortality was below 50% 48 h—post inoculation but reached 80% after 120 h of inoculation.

Positive correlation existed between the concentration of IJs and time for mortality (Table 1). It did not exist at 24 h after inoculation for *S. bicornutum* (PDBC EN 3.2) and the same trend was not positive for *H. indica* (PDBC EN 13.3) as it accounted for 6.6% mortality only. Irrespective of time, correlation was not positive for concentration of 25 IJ/larva and for *S. bicornutum* (PDBC EN 3.2). The correlation co-efficient (0.729–0.953) obtained in our study are in agreement with the findings of Morris *et al.* (1990).

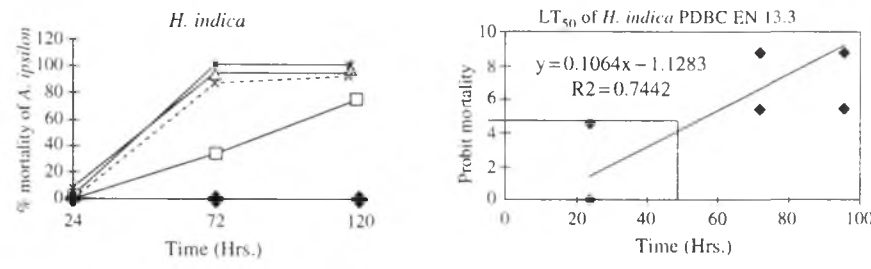

Fig. 1

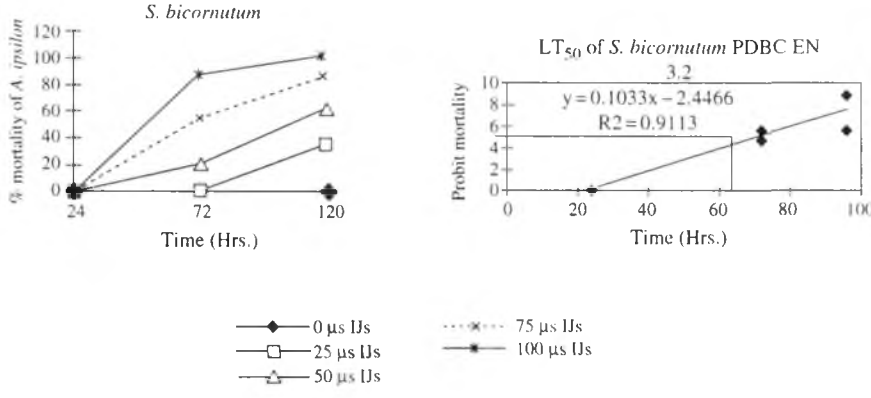

Fig. 2

FIGURE 1. Percent mortality of *A. ipsilon* larvae due to *H. indica* at different dosages.

FIGURE 2. Percent mortality of *A. ipsilon* due to *S. bicornutum* at different dosages.

Concentrations	Time	
	S. bicornutum (PDBC EN 3.2)	*H. indica* (PDBC EN 13.3)
Conc 1(0)	—	—
Conc 2(25)	0.499578	0.772055[a]
Conc 3(50)	0.729663[a]	0.862658[a]
Conc 4(75)	0.954636[a]	0.868077[a]
Conc 5(100)	0.953796[a]	0.944911[a]

[a] Table values—0.5139 (5% level of significance) and 0.6411 (1% level of significance).
Values are highly significant at 5% and 1% level of significance.

TABLE 2. LC_{50} and LC_{90} values calculated from dosage-response assays conducted with *S. bicornutum* PDBC EN 3.2 and *H. indica* PDBC EN 13.3

Nematode isolate	Incubation period (hrs)	Regression equations	R^2	LC_{50} no. of nematodes	C1 (95%) no.of nematodes	LC_{90} no.of nematodes
S. bicornutum	24	—	—	—	—	—
PDBC EN 3.2	72	$Y = 12.634X - 5.1841$	0.8387	64	59–125	81
	120	$Y = 8.5064X - 0.0265$	0.6610	39	34–105	54
H. indica	24	—	—	—	—	—
PDBC EN 13.3	72	$Y = 8.911X - 0.2557$	0.7364	34	30–89	47
	120	$Y = 8.2843 + 1.3603$	0.7581	28	24–77	39

In the dose—response assay mortality due to nematode infection was not observed till 24 h post exposure and hence not taken into consideration for calculating LC_{50}. Ricci *et al.* (1995) found that LD_{50} and LD_{90} values for 24 h after exposure could be calculated only for few species of *Sterinernema* tested. From the regression equations (Table 2) LC_{50} for *S. bicornutum* PDBC EN 3.2 was 64, 39 and 34 and 28 IJ per larvae for *H. indica* PDBC EN 13.3 at 72 and 120 h post inoculation respectively. Morris *et al.* (1990) obtained LC_{50} values of 25 and 49 for *S. feltiae* and *S. glaseri* against *A. ipsilon* and 13.8 for *H. heliothidis* against 96 h post exposure.

On comparing LC_{50} values the differences in values were more pronounced at 72 h after exposure than that of 120 h as observed by Epsky and Capinera (1994) against *A. ipsilon*. However LC_{50} values for both nematode species reduced with increase in exposure time. This supports the finding of Ricci *et al.* (1995). The minimum number of *S. bicornutum* and *H. indica* required to bring mortality of infective juveniles were 59 and 30 respectively. The LC_{90} values of *S. bicornutum* and *H. indica* were 81 and 47 IJ per larva. The LD_{90} dose caused high infection but LD_{50} caused moderate infection (Morris *et al.*, 1990). As the LC_{50} for both species were around 50 IJ per larva the LT_{50} was calculated for that concentration only by time response assay and it was found to be 72 h for *S. bicornutum* PDBC EN 3.2 and 58 h for *H. indica* PDBC EN 13.3 respectively (Fig. 2). However increase in concentration reduced the time for mortality.

The authors are thankful to the Department of Biotechnology, New Delhi for financial assistance.

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Genetic divergence among some breeds of silkworm *Bombyx mori* L.

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ABSTRACT: Assessment of genetic divergence was made among 11 parental silkworm breeds in order to know the diversity existing among them. The magnitude of all possible D^2 values ranged from 172.63 to 53 494.65 indicating very high variability. D^2 analysis of the 11 parental breeds resulted in three distinct clusters with cluster I consisting of PM, KG, KJ, HM and Nistarti; Cluster II consisting of NB_{18}, KA, P_2D_1, NP_2 and NB_4D_2 and Cluster III comprising of only one parent namely Thai. The intra cluster distance ranged from 0.00 to 1689.37 implying the prevalence of substantial amount of intracluster diversity.

KEYWORDS: genetic divergence, silkworm breeds.

INTRODUCTION

The concept of genetic distance has been of vital utility in many contexts and more so in differentiating well defined populations. Several measures of genetic distance have been proposed over years to suit various objectives in silkworm breeding.

Mahalanobis' generalized distance estimated by D^2 statistic (Rao, 1952) is unique tool for discriminating populations by considering a set of characters together, rather than inferring from indices concerning morphological similarity, eco-geographic diversity, phylogenetic relationship and similar other criteria. Bhatt (1973) conducted a comparable study of D^2 technique with other breeding methods with the objective of rationalizing the procedure of choosing parents for the hybridization. The application of D^2 statistic in finding diverse parents for hybridization was more efficient than choosing parents based on eco-geographic diversity. It has been shown that the inclusion of developmental traits that are greatly subjected to environmental variation will not affect the divergence estimates as D^2 analysis is self-weighing (Harlon, 1972).

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TABLE 1. Distribution of 11 parental breeds of silkworm in different cluster

Cluster	Breeds included	No. of breeds
I	Pure Mysore (PM)	5
	Hosa Mysore (HM)	
	Nistari	
	Kolar Gold (KG)	
	Kollegal Jawan (KJ)	
II	NB_{18}	5
	KA	
	P_2D_1	
	NP_2	
	NB_4D_2	
III	Thai	1

Moreover, inclusion of large number of traits is expected to increase the precision of estimates. Presently, the data on 13 quantitative traits were considered to be important from the point of their commercial importance to assess the diversity by adopting Mahalanobis's concept of generalized distance (D^2). The present studies were undertaken to ascertain the magnitude of genetic diversity among the different multivoltine and bivoltine silkworm breeds of *Bombyx mori* L. maintained at Department of Sericulture, University of Agricultural Sciences, Bangalore.

Seven multivoltine and four bivoltine breeds were selected for the study. All the breeds were reared in cellular replications. The rearings were conducted in a randomized block design during 1997–99, as per new technology of silkworm rearing (Krishnaswami, 1994). Observations were made on progression to fourth instar, fifth instar duration, total larval duration, mature larval weight, cocoon yield by number and weight, effective rate of rearing, cocoon and pupal weights, shell ratio percentage, productivity, rate of pupation and moth emergence, single cocoon filament length and fecundity.

Analysis of variance was done taking plot means. Based on the procedure described by Rao (1952), Mahalonobi's distance (D^2) values were calculated using the transformed uncorrelated means for 15 variables.

Assessment of genetic divergence of 11 parental silkworm breeds was made in order to study the diversity existing among them.

The correlated unstandardized means of characters were transformed into standardized uncorrelated set of variables using Dwyer's square root method (Rao, 1952). The statistical distance (Mahalanobis' D^2) between pairs of accessions was obtained as the sum of squares of differences between pairs of corresponding uncorrelated values of any two genotypes. Since each produced 10 combinations, 121 D^2 values were obtained for 11 genotypes.

In the present investigation, the magnitude of all possible D^2 values ranged from 172.63 to 53 494.65 indicating very high variability. The 11 parents were grouped into

TABLE 2. Intra and inter-cluster average divergence (D^2) values of three clusters of silkworm breeds

	Cluster		
	I	II	III
I	1689.37	14783.80	7217.48
II		1376.79	39573.71
III			0.00

three clusters. The intra cluser distance ranged from 0.00 to 1689.37 implying the prevalence of substantial amount of intra-cluster diversity (Table 2).

It is a general notion that larger the divergence between the genotypes, higher will be the heterosis. In the present study intercluster distance between Cluster I and II was largest followed by that between I and III. Therefore, genotypes from these clusters can be selected for hybridization purpose to evolve higher heterotic crosses which might prove potential for isolating superior seggregants in advanced generations. Selection of parents based on the extent of genetic divergence has been successfully adopted in different crop species (Swaminathan, 1979).

Apart, from selecting lines from the clusters which have high intercluster distance for hybridization one can also think of selecting parents based on the percent character contribution towards total divergence depending upon the specific objective of the breeder. For instance, if a breeder intends to improve upon cocoon yield, parents which are highly divergent with respect to that character can be selected. As per Subbarao *et al.* (1989), the genotypes with significant differences should be selected as parents for diallel analysis and silkworm hybridization.

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A new species of *Aleurocanthus* and *Asialeyrodes indica* Sundararaj & David (Aleyrodidae: Homoptera) from Andaman & Nicobar Islands

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ABSTRACT: A new species of *Aleurocanthus* collected from banana (*Musa* sp.) in Andaman & Nicobar Islands has been described as *Aleurocanthus musae* and illustrated. The occurrence of *Asialeyrodes indica* Sundararaj & David on *Ervatania coronaria* has been reported.

KEYWORDS: Aleyrodidae, *Aleurocanthus musae*, *Asialeyrodes indica*, Andaman & Nicobar Islands.

Little is known about the whitefly fauna of the Andaman & Nicobar Islands. In the early 1990s the senior author had examined specimens of *Bemisia tabaci* (Gennadius) collected from brinjal (*Solanum melongena*) in Port Blair. During April 2001 BVD collected whiteflies from the Andaman & Nicobar Islands. In this paper a new species of *Aleurocanthus* occurring on banana (*Musa* sp.) is described and illustrated. In addition occurrence of *Asialeyrodes indica* Sundararaj & David in Andaman & Nicobar Islands has been reported for the first time.

***Aleurocanthus musae* sp. nov.**

Description

Pupal case Pale yellowish brown covered with wax; found in groups on the under surface of leaf. Elliptic in outline and widest across second abdominal segment. Measures 0.97–0.99 mm long and 0.59–0.60 mm wide.

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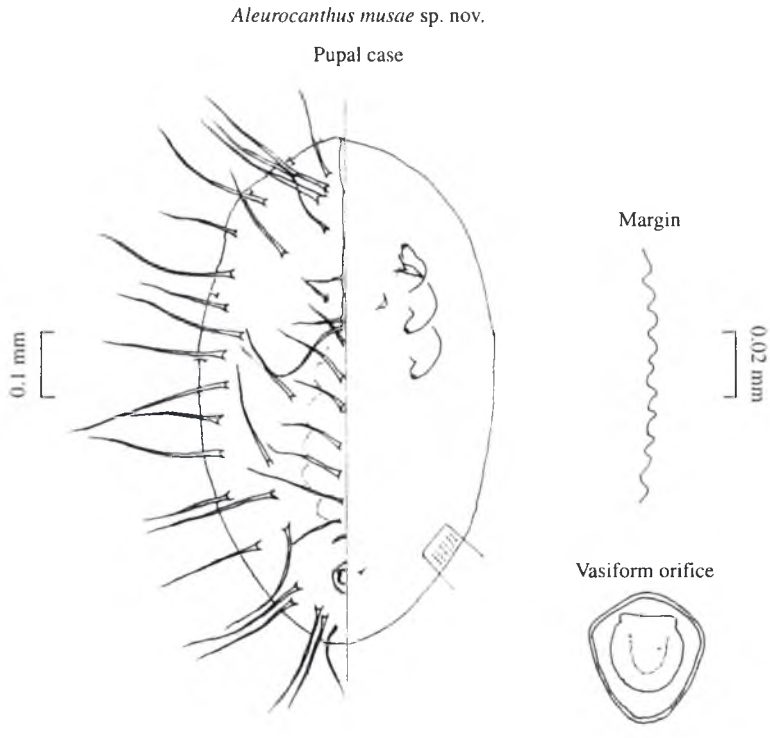

Margin Irregularly crenate, 8–9 crenations in 0.1 mm; thoracic and caudal tracheal pore areas not differentiated. Anterior marginal setae 22 μ long.

Dorsum Prothorax large, meso- and meta-thorax small. Abdominal segments not distinctly discernible. Dorsal setae not noticeable except caudal setae, 0.19 mm long. Four submarginal pointed setae—3 in cephalothorax and 1 laterad of second abdominal segment, 31–42 μ long. Twelve pairs of spines noticed on cephalothorax—6 on submargin, 2 on subdorsum and 4 positioned medially. Twenty one pairs of abdominal spines present: 10 pairs on submargin, 4 subdorsally laterad of abdominal segments 2, 5, 6 & 7, and 7 pairs medially on abdominal segments 1–7. Submarginal spines 0.12–0.26 mm long; subdorsal spines 0.23–0.25 mm long; and median spines 0.11–0.19 mm long. Rhachis faintly present on abdomen. Longitudinal suture reaches margin, and transverse moulting suture descends down and moves upward to reach subdorsum.

Vasiform orifice Elevated, cordate, 60–72 μ long and 53–55 μ wide. Operculum sub quadrate, fills orifice concealing lingual. Caudal furrow absent.

Venter Ventral abdominal setae 55 μ long and 60 μ apart. Thoracic and tracheal folds wanting. Four to five rows of irregular sculpturing along submargin except in thoracic and caudal tracheal fold regions. Antenna 35 μ long reaching base of prothoracic leg. Spines at base of legs wanting.

Holotype One pupal case on slide; Wandoor, Andaman & Nicobar Islands, *Musa* sp., 25.04.2001, B.V. David. Deposited in the collections of the Division of Entomology, Indian Agricultural Research Institute, New Delhi.

Paratypes Three pupal cases mounted on slides, bearing same details. One deposited in the collections of RWA. Madras Christian College and two with BVD.

This species is close to *Aleurocanthus davidi* David & Subramaniam and *A. indicus* David & Regu in possessing minute submarginal setae but differ from them in possessing 33 pairs of dorsal spines as against respectively 22 and 24 pairs. The only species of *Aleurocanthus* reported to occur on *Musa* spp. is *A. woglumi* Ashby (Mound and Halsey, 1978). However, *A. woglumi* is quite different from the new species described here.

***Asialeyrodes indica* Sundararaj & David**

Asialeyrodes indica Sundararaj & David. 1991, *J. Bombay Nat. Hist. Soc.*, **88**: 413.

This species was first reported from India in 1991 infesting *Ervatomia coronaria* in Coimbatore (Tamil Nadu), Ambalamedu (Kerala) and Mumbai (Maharashtra). It was found infesting *Ervatomia coronaria* in Andaman & Nicobar Islands which is a new record for this region.

Material examined Five specimens mounted on slides; collected from *Ervatomia coronaria*, 25.iv.2001, Wandoor (Andaman & Nicobar Islands), Coll. B.V. David.

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Further studies on two Indian species of genus *Sidyma* Walker (Lithosiinae: Arctiidae: Lepidoptera)

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ABSTRACT: The taxonomically important features including the male and female genitalia of two Indian species i.e. *Sidyma albifinis* Walker and *S. apicalis* Moore have been studied and illustrated. The genus diagnosis has been revised. A key to both these known Indian species has also been formulated.

KEYWORDS: *Sidyma*, *albifinis*, *apicalis*, *genitalia*.

While conducting surveys in different localities of North and North-eastern states for the collection of footman moths, as many as fourteen individuals of two species referable to genus *Sidyma* Walker have been collected. Both these species have been identified as *S. albifinis* Walker and *S. apicalis* Moore with the help of relevant literature (Hampson, 1894) and their identity has been confirmed from Zoological Survey of India, Calcutta and Forest Research Institute, Dehradun. Hampson (1894) described two species of the present genus under Sidyma group and four species from Sikkim under Vamuna group. The generic name *Vamuna* Moore has already been brought into use with the type species *remelana* Moore by Watson *et al.* (1980). At present, the genus *Sidyma* Walker is represented by both the species studied here. The morphological features such as labial palpi, antennae, wing venation & maculation including the male and female genitalic characters have been studied here in detail. The genitalic features have been studied and illustrated for the first time and incorporated in the genus diagnosis. The critical study of these features revealed that both these species are clearly congeneric and form a natural group.

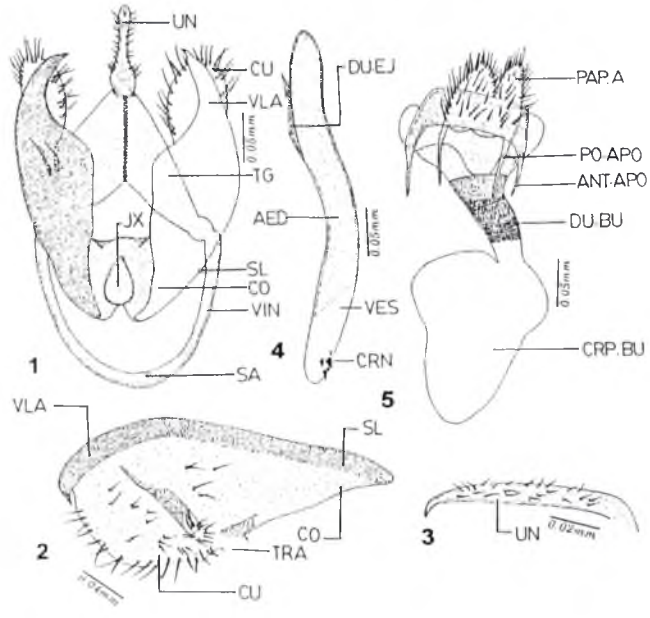
OBSERVATIONS

Genus Sidyma Walker

Walker, 1856, *Cat. Lep. Het.* **7**: 1686

Type species : *S. albifinis* Walker
Distribution : Himalayas, Khasi hills

PLATE 1



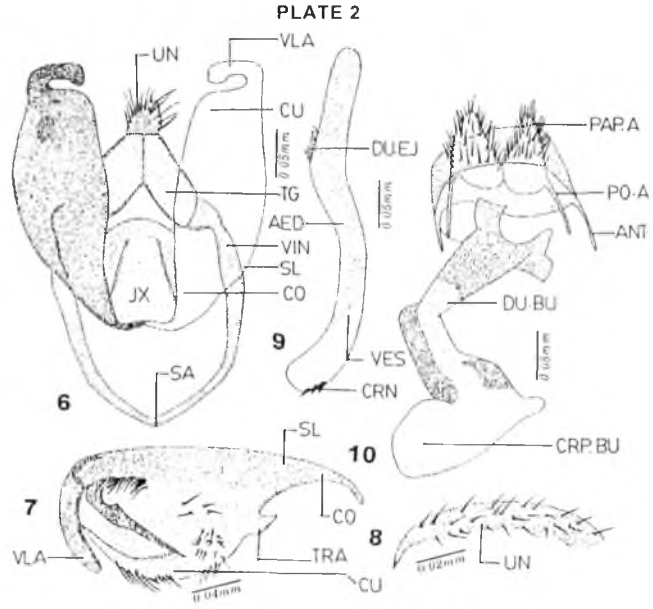
FIGURES 1–4. Male genitalia of *Sidyma albifinis* Walker. 5. Female genitalia of *S. albifinis* Walker.

Diagnosis

Labial palpus upturned, reaching just vertex of head, third joint short. Antenna bipectinate in male, ciliated in female. Forewing with veins R_2 and R_5 originating from aerole formed by anastomosis of R_3; M_1 from upper angle or from aerole; M_2 and M_3 from lower angle of cell; Cu_1 from before lower angle of cell; Cu_2 from well beyond middle of cell. Hindwing with vein Sc + R_1 arising from before middle of cell; Rs & M_1 from upper angle of cell; M_2 and M_3 stalked from lower angle of cell; Cu_1 from before lower angle of cell. Male genitalia with uncus simple, moderately long, tip pointed; tegumen broad, as long as vinculum or may be slightly longer; vinculum highly developed; saccus reduced; valva narrow at base, broad above; sacculus and costa defined, valvula curved distally, well sclerotized, cucullus broad, a sclerotized projection present, aedeagus long and curved, anterior tip rounded; vesica armed with prominent cornuti. Female genitalia with corpus bursae large and membranous; signum absent; ducturs bursae membranous and narrow at anterior end, well sclerotized and with broad lower half; papilla analis broad and triangular.

Key to the species of Genus *Sidyma* Walker

1. Hindwing with a white apical patch, vein Cu_1 arising from just before lower angle of cell; male genitalia with valvula curved distally, dentate margins



FIGURES 6–9. Male genitalia of *S. apicalis* Moore. 10. Female genitalia of *S. apicalis* Moore.

prominent, tip pointed; tegumen rather broad; vesica armed with five cornuti ..... *albifinis* Walker.

..... Hindwing without any apical patch, vein Cu_1 originating well before lower angle of cell; male genitalia with valvula without dentate margins, tip like snake's hood; tegumen not broad; vesica armed with three cornuti ..... *apicalis* Walker.

Sidyma albifinis (Walker)

Walker, 1856, *Cat. Lep. Het.* **7**: 1686; Hampson. 1894,*Moths Ind.* **2**: 67

Male genitalia: Uncus moderately long, swollen at base, with a slightly curved, sharp pointed tip; tegumen broad, as long as vinculum; vinculum V-shaped, highly developed; saccus reduced. Valva narrow at base, broad above, sacculus and costa defined; valvula curved distally, margins dentate, pointed at tip; cucullus broad, with a sclerotized flap-like projection at base, rounded at distal end; juxta moderately sclerotized, conical; transtilla marked. Aedeagus long and curved, anterior tip rounded, vesica armed with five cornuti (three large and two small) at distal end.

Female genitalis: Corpus bursae broad and membranous; signum absent; ductus bursae membranous and narrow at anterior end, strongly sclerotized and broad lower half; apophyses narrow with their apices pointed; papilla analis broad and rounded, fringed with micro and macro setae.

Wing expanse (half)	:	Male : 21 mm Female : 22 mm
Material examined	:	Himachal Pradesh; Narkanda, 27.06.1995, 1 ♂, 28.06.1995, 1 ♂, Kharapatuar, 20.06.2000, 2 ♂♂; Darang, 24.06.2000, 1 ♂–Uttar Pradesh: Chakrata, 15.06.1993, 3 ♂♂, 3 ♀♀; Nanital, 29.06.1994, 1♂.
Old distribution	:	N.W. Himalayas

***Sidyma apicalis* Moore**

Moore, 1878, P. Z. S. 1878: 9; Hampson, 1894, *Moths Ind.* **2**: 67

Male genitalia: Uncus moderately long, curved, swollen at base and sharply pointed, tip beak-like; tegumen broad and triangular; vinculum longer than tegumen, narrow, U-shaped; saccus reduced. Valva narrow anteriorly, broad posteriorly, sacculus and costa marked; valvula extending into a curved projection with its tip resembling that of snake's hood; cucullus broad, round with a large flap extending along whole width of valva; juxta clypeiform; transtilla triangular. Aedeagus long with its tip rounded, anterior half straight, posterior half curved; vesica armed with three spine-like cornuti at distal end.

Female genitalia: Corpus bursae globular, membranous; signum absent; ductus bursae globular, membranous; signum absent; ductus bursae broad and semisclerotized anteriorly, narrow and strongly sclerotized posteriorly; both pair of apophyses long with their tips narrow; papilla analis triangular and rounded, setosed with small and large setae.

Wing Expanse (Half)	:	Male : 21 mm : Female : 20–22 mm
Material examined	:	West Bengal: Kurseong, 28.04.1995, 2 ♀♀; 29.04.1995, 1 ♂, 2 ♀♀.
Old distribution	:	Sikkim

Remarks

Reporting of this species from Kurseong is its new record from West Bengal state.

Abbreviations

AED	:	Aedeagus	PAP. A	:	Papilla Analis
ANT. APO	:	Anterior Apophyses	PO. APO	:	Posterior apophyses
CEB. BU	:	Cervix Bursae	SA	:	Saccus
CO	:	Costa	SL	:	Sacculus
CRN	:	Cornuti	TG	:	Tegumen
CRP. BU	:	Corpus Bursae	TRA	:	Transtilla
CU	:	Cucullus	UN	:	Uncus
DU. BU	:	Ductus Bursae	VES	:	Vesica
DU. EJ	:	Ductus Ejaculatoris	VIN	:	Vinculum
JX	:	Juxta	VLA	:	Valvula

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Additional notes on a Himalayan Satyrid, *Hipparchia parisatis* (Kollar) (Lepidoptera: Satyridae)

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ABSTRACT: *Hipparchia parisatis* (Kollar), a highly variable taxon, collected from different localities in the Western Himalaya has been dealtwith in the present communication. The distribution of this species has been recorded to show the prevalence in the existing environment. The population variations and conspecificity aspects have also been authenticated on the basis of genitalic studies.

INTRODUCTION

Recently while working on the taxonomy of the genus *Hipparchia* Fabricius, Kudrna (1977) drastically reduced two hundred and ten specific/subspecific names hitherto known under this genus to merely forty-two names with a remark that many earlier works amounted to senseless mania (Marshall and de Niceville, 1883; Bingham, 1905; Evans, 1932; Talbot, 1947; Mani, 1986). In view of this, the species *parisatis* kollar under the genus *Eumenis* Hubner by Evans (loc. cit.) and Wynter-Blyth (1957) has been held as a wrong and accordingly the same is presently dealtwith under *Hipparchia* as *H. parisatis* (Kollar) (Kudrna, loc, cit.).

Like many other satyrid species, *Hipparchia parisatis* (Kollar) too is a highly variable taxon. Owing to this, during the course of present studies, a phenon comprising eighteen individuals (12 males and 6 females) collected from various localities in Western Himalaya has been examined. Though Kudrna (1977) has examined, as many as, five hundred and five specimens from different museums with a view to record various variations viz-a-viz to straighten the taxonomic position of this species, yet some additional variations have been presently recorded and the same are given in Table 1.

TABLE 1. Showing some additional variations in *Hipparchia parisatis* (Kollar)

Sl. No.	Taxonomic character	Earlier Account (Kudrna, 1977)	Present observations
1.	Forewing (upperside)	(i) Uniform blackish-brown (6 males, 1 female), with white outer margin (8 males, 3 females) (ii) Black subapical ocellus with white-pupil (6 males, 6 females)	(i) Brown (6 males, 5 females), with outer margin obscure (4 males, 3 females) (ii) Black subapical ocellus without white-pupil (6 males) (iii) Two post-discal white dots prominent (3 males, 6 females) or obscure (9 males)
2.	Hindwing (upperside)	(i) Black spot or black ocellus with white pupil near tornus (9 males, 6 females)	(i) Black spot or black ocellus with white-pupil wanting (3 males)
3.	Forewing (underside)	(i) One or two ocelli consisting of concentric yellow and black circles with white pupil	(i) Subapical black ocellus consisting of concentric yellow and black circles with white pupil (12 males, 6 females) (ii) Subtornal black ocellus consisting of concentric yellow and black circles without white pupil (8 males, 6 females (iii) Subtornal ocellus obscure (4 males)
4.	Hindwing (underside)	(i) Two well marked submarginal ocelli similar to those on forewing	(i) Subapical and subtornal ocelli may be equal sized (6 males, 3 females) or unequal sized (6 males, 3 females) (ii) Subtornal ocellus without white centre (one male)

MATERIAL EXAMINED

Himachal Pradesh: 2♂, 3♀, 14.vi.91, Sarol, Chamba; 3♂, 1♀, 17.vi.91, Rakh, Chamba; 2♂, 25.vi.96, Sanz, Shimla; 3♂, 14.vi.96, Taklech, Shimla; 1♂, 11.vi.96, Solan; 1♂, 29.v.92, Pahar View, Kasauli, Solan: 1♀, 21.vi.95, Manikaran, Kullu.

Uttranchal: 1♀, 12.vi.95, Water Falls, Chakrata.

It need hardly be emphasised that various species belonging to the genus *Hipparchia* Fabricius are highly variable and all such variable individuals were named as independent species/subspecies and the number of such taxa rose upto as high as two hundred and forty two (Kudrna, 1977). All such names have consistently been used in various publications (Marshall and de Niceville, 1883; Bingham, 1905; Evans, 1932; Talbot, 1947; Mani, 1986). As many as, two hundred and forty two names have been given to variable individuals of *parisatis* kollar which according to Kudrna (loc. cit.) contain no subspecies at the moment. Gross and Ebert (1976) have considered the white margin of the forewings to be of taxonomic significance in *Satyrus parisatis ismail* Gross & Ebert. However, the present study shows that this character too present variations in both sexes. Owing to these and other variations, four males and two females have been dissected and have been found to be highly conspecific. Kudrna (loc. cit.) had mentioned that this clinal character of geographical variation of *parisatis* Kollar accounts for new synonyms proposed by him.

The collection of the species from Taklech (1600 m, Shimla) is a new distributional record in the Western Himalaya. It has earlier been reported from localities such as, Mussoorie (2005 m), Shimla (2100 m), Pangi (2750 m) and Bajoura (1220 m, Kullu) (Marshall and de Niceville, 1883; Mackinnon and de Niceville, 1897; Kudrna, 1977).

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Record of leaf twisting weevil (*Apoderus sissu* Marshall) as a pest on rose

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In India, rose (*Rosa indica* Linn.) is infested by about 15 species of insects pests besides damage caused by mites and nematodes (Pal, 1991; Vyas, 1998). Recently, incidence of a leaf twisting weevil *Apoderus sissu* Marshall (Fig. 1) was recorded from rose at Cardamom Research Station, Pamapdumpara, Idukki, Kerala for the first time.

FIGURE 1. Rose leaves fed by leaf twisting weevil and adult weevil (inset).

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The adult weevils which are free living, are found to scrap the tender rose leaves that are pink in colour skeletonizing them in patches. The cream coloured eggs that are 1.5 mm long are laid singly into a roll of the leaf which protects the newly laid egg from being predated. After hatching, the leaf is fed by the grub from within. The grub and pupal stages are completed within the leaf roll and the adult emerged in 20–25 days through a hole made on the side of the roll. The damage is very severe during the summer months (March to May). Incidence of this insect pest was observed during past two years in high range rose gardens of Kerala. Not all varieties of rose are infested by the weevil. Among 520 accessions screened, VL-20 rose variety is highly susceptible and this variety retained pink colour at tender leaf stage for a longer period of time. Application of Monocrotophos (0.05%) is very effective in suppressing the insect population and damage.

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